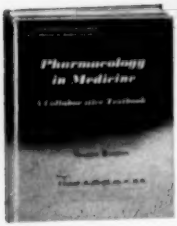
THE JOURNAL OF

MEDICAL EDUCATION

OFFICIAL PUBLICATION OF
THE ASSOCIATION OF AMERICAN MEDICAL COLLEGES

APRIL 1959 • VOLUME 34 • NUMBER 4

5 Outstanding books *from* McGraw-Hill



PRINCIPLES OF INTERNAL MEDICINE—3rd Edition

Edited by T. R. Harrison, A.B., M.D.; R. A. Adams, B.A., M.A., M.D., M.A. (Hon.); I. L. Bennett, Jr., A.B., M.D.; W. H. Resnik, M.D., Ph.B.; G. W. Thorn, M.D., M.A. (Hon.), LL.D. (Hon.), Sc.D. (Hon.); M. M. Wintrobe, B.A., M.D., B.Sc. (Med.), Ph.D.

1782 pp., plus index, 7½ × 10

One volume edition *$18.50*
Two volume edition (not shown) *$24.00*

This new edition contains a vast amount of NEW information. For example, Part Two, Cardinal Manifestations of Disease, has been thoroughly revised. The sections dealing with disorders of circulatory and pulmonary function have been rewritten in order to bring them into line with rapid advances in these fields.

PHARMACOLOGY IN MEDICINE—2nd Edition

Edited by Victor A. Drill, Ph.D., M.D.

1273 pp., 8½ × 11, illus., $19.50

The work deals with those drugs currently in practical use with emphasis on their mechanism of action, effect within the body, therapeutic value, and available forms. Indexed according to chemical constituents as well as by trade name. Physiologic action of drugs is stressed. This 2nd Edition contains a new chapter on drugs affecting behavior.

NEW REVISIONS—THIS MONTH

SURVEY OF CLINICAL PEDIATRICS—3rd Edition

By Lawrence B. Slobody, M.D.

The growth and changing practices of pediatrics have necessitated this early revision of the third edition, and every phase of the book has been extensively revised and brought up to date. All the material presented has been used with the student in actual work at the bedside and in conference. As in previous editions, the author emphasizes strongly that the physical, mental, emotional, and social aspects of the child are indivisible and require consideration.

ANALYTICAL CYTOLOGY—2nd Edition

By Robert C. Mellors, M.D., Ph.D.

This book presents the theory and practical applications of some of the physical and chemical methods presently used in the analysis of cellular structure and function. New chapter on technique and application of fluorescent antibody method has been added. The chapter of intracellular location of chemical constituents integrates biochemistry and biomorphology. Also added is new information on electron microscopy to keep abreast with the rapid advances in this field.

PRINCIPLES OF BIOCHEMISTRY—2nd Edition

By A. White, Ph.D.; P. Handler, Ph.D.; E. L. Smith, Ph.D.; D. Stetten, Jr., M.D., Ph.D.

1048 pp., plus index, illus., $15.00

A basic, modern textbook of biochemistry, this work emphasizes the fundamental principles and concepts of the subject, with consideration to the mechanisms of, and factors influencing, biological reactions where they are known. Chapters on metabolism and endocrinology have been completely rewritten in line with the rapid advances in these fields.

50 YEARS OF PUBLISHING · McGRAW-HILL BOOK COMPANY · 1909-1959

THE JOURNAL OF MEDICAL EDUCATION

Official Publication of the Association of American Medical Colleges

EDITORIAL OFFICE

University Hospitals, School of Medicine, University of Wisconsin, Madison 6, Wisconsin, ALpine 6-6811

JOHN Z. BOWERS, *Editor-in-Chief*
ELIZABETH B. POHLE, *Assistant Editor*

OFFICE OF THE ASSOCIATION OF AMERICAN MEDICAL COLLEGES

2530 Ridge Avenue, Evanston, Illinois, DAvis 8-9505

TOM COLEMAN, *News Editor*
NEVA V. RESEK, KAY MCDONOUGH, *Assistants*
MARY E. PARRILLI, *Advertising*

INFORMATION FOR SUBSCRIBERS

The Journal of Medical Education is owned by the Association of American Medical Colleges and published monthly by the University of Chicago Press, 5750 Ellis Avenue, Chicago 37, Illinois. Second-class postage paid at Chicago, Illinois.

Subscription Rates: $7.00 per year, $13.50 two years, $19.50 three years, $1.00 per single copy; foreign, $8.00 per year, $15.50 two years, $22.50 three years, $1.25 per single copy; Pan America and Canada, $7.50 per year, $14.50 two years, $21.00 three years. Supplements, $2.00.

INFORMATION FOR CONTRIBUTORS

The Journal of Medical Education serves as an international medium for the exchange of ideas in medical education, as well as a means of communicating the policies, programs, and problems of the Association. The Editorial Board welcomes the submission of manuscripts concerned with the broad field of medical education; this includes preparation for medical education; the medical school experience; intern and resident education; graduate and postgraduate medical education. The Editorial Board recognizes that medical education includes the activities of faculty, students, administrators, and those of the practicing profession who also teach and learn. Thus, it invites communications from any of these sources.

Manuscripts should be submitted in duplicate. All manuscripts are reviewed by the Editorial Board before acceptance for publication. All copy, including footnotes, tables, and legends, should be typed double-spaced. Each diagram or graph or photograph should have a brief legend. Each table should be typed on a separate sheet of paper. References should refer to published material only, must be submitted in alphabetical order, and should include, in order: author, title, journal abbreviation (*Quarterly Cumulative Index Medicus* form), volume number, page, and year; book references should also include editors, edition, publisher, and place of publication.

Galley proofs will be mailed to authors for correction before publication and should be returned within 48 hours after receipt.

Reprints may be ordered, when galley proofs are returned, from the University of Chicago Press, in multiples of 50, at a price depending on the length of the article; prices are listed on the reprint order form.

Medical Education Forum includes editorials, letters, comments, criticisms, and excerpts from important addresses.

News from the Medical Schools: Material for this section should be transmitted to the News Editor, Mr. Tom Coleman, 2530 Ridge Avenue, Evanston, Illinois. Announcements of major faculty and administrative appointments, news of distinguished visitors and significant educational developments will be included. It is not possible to publish notices on grants-in-aid for scientific research.

Items of Current Interest: Audio-visual news and notices from national and federal agencies appear in this section.

CORRESPONDENCE

Address all correspondence regarding manuscripts, editorials, and letters to the Editor to the Editorial Office, University Hospitals, University of Wisconsin, Madison 6, Wisconsin.

Address all correspondence concerning subscriptions, reprints, changes of address, and back numbers to the University of Chicago Press, 5750 Ellis Avenue, Chicago 37, Illinois. All changes of address should provide both the old and the new address.

Address all correspondence concerning news, announcements, and personnel exchange to the office of the Association of American Medical Colleges, % Mr. Tom Coleman, 2530 Ridge Avenue, Evanston, Illinois; address all correspondence concerning advertising to Mrs. Mary E. Parrilli, 2530 Ridge Avenue, Evanston, Illinois.

PRINTED IN U.S.A

NOW EVEN BETTER!

BAUSCH & LOMB
Spectronic 20
COLORIMETER

● **IT'S TODAY'S MOST USEFUL COLORIMETER** From the near infrared to near ultra-violet . . . you get wider range coverage with the Spectronic 20 than with any other colorimeter at any price. It does tests no other colorimeter can handle . . . like transaminase, at 340mμ; Vitamin E in Blood, at 725mμ; literally hundreds of others . . . fast and easy as tuning your radio. Highest accuracy, of course.

● **IT'S TODAY'S LOWEST-COST SPECTROPHOTOMETER.** Colorimeter *plus* spectrophotometer in one compact unit . . . unequalled at twice the price. Dial instantly sets Certified-Precision Grating to desired wavelength; no filters to fuss with. 340-950mμ range, $281. 375-950mμ $261. 375-650mμ, $255.

● **. . . AND NOW IT'S A BETTER BUY THAN EVER!** How can the best be made even better? Count on B&L to keep trying. Now, at no increase in price, the Spectronic 20 features more precise sample positioning that provides even higher stability and reproducibility. Try it out in your lab, in an obligation-free demonstration.

OVER 9000 IN DAILY USE . . .
MAIL COUPON TO FIND OUT WHY

BAUSCH & LOMB OPTICAL CO.
14740 St. Paul St., Rochester 2, N. Y.

☐ Send me B&L Spectronic 20 Catalog D-266.

☐ Schedule demonstration at my convenience.

NAME .....

TITLE .....

PROFESSIONAL ADDRESS .....

.....

Quartier Latin

"A student's room in the Hôtel de l'Avenir. . . . Mornings in the wards of La Salpêtrière, Hôtel-Dieu and La Pitié, going from bed to bed to read chapter after chapter in the book of human suffering, written with blood and tears. Afternoons in the dissecting rooms and amphitheatres of l'École de Médecine or in the laboratories of the Institut Pasteur, watching in the microscope with wondrous eyes the mystery of the unseen world. . . . Nights of vigil in the Hôtel de l'Avenir, precious nights of toil to master the hard facts, the classical signs of disorder and disease collected and sifted by observers from all lands. . . . Then came spring and dropped the first twig of chestnut flowers on my balcony from the budding trees of the avenue. It was the signal. I went up for my exam and left the Hôtel de l'Avenir with the hard-won diploma in my pocket, the youngest M.D. ever created in France."

— Axel Munthe (*The Story of San Michele*, 1929)

Calendar of Meetings

ASSOCIATION OF AMERICAN MEDICAL COLLEGES

70th Annual Meeting, November 2–4

Edgewater Beach Hotel, Chicago, Ill.

MAY

AMERICAN ASSOCIATION FOR THE HISTORY OF MEDICINE, Wade Park Manor, Cleveland, May 21–23. Dr. John B. Blake, Smithsonian Institution, Washington 25, D.C., Secretary.

AMERICAN COLLEGE OF CARDIOLOGY, Benjamin Franklin Hotel, Philadelphia, May 26–29. Dr. Philip Reichert, 480 Park Ave., New York 22, Secretary.

AMERICAN FEDERATION FOR CLINICAL RESEARCH, Chalfonte-Haddon Hall, Atlantic City, N.J., May 3. Dr. George E. Schreiner, Georgetown Univ. Hosp., Washington 7, D.C., Secretary.

AMERICAN GYNECOLOGICAL SOCIETY, The Homestead, Hot Springs, Va., May 21–23. Dr. Andrew A. Marchetti, 3800 Reservoir Rd., N.W., Washington 7, D.C., Secretary.

AMERICAN OPHTHALMOLOGICAL SOCIETY, The Homestead, Hot Springs, Va., May 28–30. Dr. Maynard C. Wheeler, 30 West 59th St., New York 19, Secretary.

AMERICAN PEDIATRIC SOCIETY, The Inn, Buck Hill Falls, Pa., May 6–8. Dr. A. C. McGuinness, 2800 Quebec St., Washington 8, D.C., Secretary.

AMERICAN SOCIETY FOR CLINICAL INVESTIGATION, Haddon Hall, Atlantic City, N.J., May 3–4. Dr. S. J. Farber, 550 1st Ave., New York 16, Secretary.

AMERICAN TRUDEAU SOCIETY, Palmer House, Chicago, May 25–27. Dr. E. P. K. Fenger, 1790 Broadway, New York 19, Secretary.

ASSOCIATION OF AMERICAN PHYSICIANS, Haddon Hall, Atlantic City, N.J., May 5–6. Dr. Paul B. Beeson, Yale Univ. School of Medicine, New Haven 11, Conn., Secretary.

MISSISSIPPI STATE MEDICAL ASSOCIATION, Hotel Buena Vista, Biloxi, May 12–14. Mr. Rowland B. Kennedy, 735 Riverside Dr., Jackson, Executive Secretary.

NATIONAL TUBERCULOSIS ASSOCIATION, Palmer House, Chicago, May 24–29. Mrs. Wallace B. White, 1790 Broadway, New York 19, Secretary.

SOCIETY OF AMERICAN BACTERIOLOGISTS, Sheraton-Jefferson Hotel, St. Louis, May 10–15. Dr. E. M. Foster, University of Wisconsin, Madison 6, Wis., Secretary.

SOCIETY FOR PEDIATRIC RESEARCH, The Inn, Buck Hill Falls, Pa., May 8–9. Dr. Clark D. West, Children's Hosp., Cincinnati 29, Secretary.

STUDENT AMERICAN MEDICAL ASSOCIATION, Morrison Hotel, Chicago, Apr. 30–May 3. Mr. Russell F. Staudacher, 430 N. Michigan, Chicago 11, Executive Secretary.

JUNE

AMERICAN ACADEMY OF TUBERCULOSIS PHYSICIANS, Atlantic City, N.J. June 6. Dr. Oscar S. Levin, P.O. Box 7011, Denver 6, Secretary.

AMERICAN COLLEGE OF ANGIOLOGY, World Conference on Angiology. Marborough Blenheim Hotel, Atlantic City, N.J. June 5–7. Dr. Alfred Halpern, 11 Hampton Court, Great Neck, N.Y., Executive Secretary.

AMERICAN COLLEGE OF CHEST PHYSICIANS, Atlantic City, N.J. June 3–7, Mr. Murray Kornfeld, 112 E. Chestnut St., Chicago 11, Executive Director.

AMERICAN DERMATOLOGICAL ASSOCIATION, Claridge Hotel, Atlantic City, N.J., June 1–4. Dr. Wiley M. Sams, 25 Southeast 2nd Ave., Miami, Fla. Secretary.

AMERICAN DIABETES ASSOCIATION, Chalfonte-Haddon Hall, Atlantic City, N.J., June 6–7, Dr. E. Paul Sheridan, 1 East 45th St., New York 17, Secretary.

AMERICAN ELECTROENCEPHALOGRAPHIC SOCIETY, Claridge Hotel, Atlantic City, N.J., June 11–14, Dr. Jerome K. Merlis, University Hospital, Baltimore 1, Secretary.

AMERICAN GASTROENTEROLOGICAL ASSOCIATION, Claridge Hotel, Atlantic City, N.J., June 5–6. Dr. Franz J. Ingelfinger, 69 E. Newton St., Boston 18, Secretary.

AMERICAN GERIATRICS SOCIETY, Hotel Traymore, Atlantic City, N.J. June 4–5. Dr. Richard J. Kraemer, 2907 Post Rd., Warwick, R.I. Secretary.

AMERICAN MEDICAL ASSOCIATION, Traymore Hotel, Atlantic City, N.J. June 8–12, Dr. F. J. L. Blasingame, 535 N. Dearborn St., Chicago 10, Executive Vice-President.

AMERICAN MEDICAL WOMEN'S ASSOCIATION, Sheraton Ritz Carlton Hotel, Atlantic City, N.J., June 4–7, Miss Lillian T. Majally, 1790 Broadway, New York 19, Executive Secretary.

AMERICAN NEUROLOGICAL ASSOCIATION, Claridge Hotel, Atlantic City, N.J., June 15–17. Dr. Charles Rupp, 133 S. 36th Street, Philadelphia 4, Secretary.

AMERICAN ORTHOPEDIC ASSOCIATION, Lake Placid Club, Lake Placid, N.Y. June 16–18. Dr. Lee Ramsey Straub, 535 E. 70th St., New York, 21, Secretary.

AMERICAN PHYSICAL THERAPY ASSOCIATION. Hotel Leamington, Minneapolis. June 21–26. Miss Annetta Cornell Wood, 1790 Broadway, New York 19, Executive Director.

AMERICAN PROCTOLOGIC SOCIETY, Shelburne Hotel, Atlantic City, N.J., June 15–18. Dr. Norman D. Nigro, 10 Peterboro St., Detroit 1, Secretary.

SOUTH DAKOTA STATE MEDICAL ASSOCIATION. Sheraton Johnson Hotel, Rapid City, June 20–23. Mr. John C. Foster, 300, 1st National Bank Bldg., Sioux Falls, Executive Secretary.

JULY

ROCKY MOUNTAIN CANCER CONFERENCE, Brown Palace Hotel, Denver. July 22–23. Dr. N. Paul Isbell, 835 Republic Bldg., Denver 2, Chairman.

AUGUST

AMERICAN HOSPITAL ASSOCIATION, Statler Hotel, New York City, Aug. 24–27. Dr. Edwin L. Crosby, 18 E. Division St., Chicago, Director and Secretary.

NATIONAL MEDICAL ASSOCIATION, Detroit, Aug. 10–13. Dr. John T. Givens, 1108 Church St., Norfolk, Va., Secretary.

INTERNATIONAL AND FOREIGN

JUNE

CANADIAN FEDERATION OF BIOLOGICAL SOCIETIES (Canadian Physiological Society, Pharmacological Society of Canada, Canadian Association of Anatomists, Canadian Biochemical Society), University of Toronto, Toronto, Ont., Canada. June 9–11. Dr. E. H. Bensley, Room 710, The Montreal General Hospital, Montreal 25, Que.

INTERNATIONAL HOSPITAL CONGRESS, Edinburgh, Scotland, June 1–6, Capt. J. E. Stone, 34 King St., London, E.C.2, England. Secretary-General.

JULY

BRITISH MEDICAL ASSOCIATION, Edinburgh, Scotland, July 18–24. For information address: The Secretary, British Medical Association, Tavistock Square, London, W.C.I., England.

CANADIAN MEDICAL ASSOCIATION, Edinburgh, Scotland, July 18–24. Dr. A. D. Kelly, 150 St. George St., Toronto 5, Ont., General Secretary.

AUGUST

INTERNATIONAL CONGRESS FOR THE HISTORY OF SCIENCE. Barcelona & Madrid Spain. Aug. 30–Sept. 6. Prof. J. Vernet, Universidad de Barcelona, Barcelona, Spain, Secretary-General.

INTERNATIONAL CONGRESS OF PHYSIOLOGICAL SCIENCES, Buenos Aires, Argentina. Aug. 9–15. A.O.M.Stoppani, Facultad de Ciencias Medicas, Paraguay 2151, Buenos Aires, Argentina.

WORLD CONFERENCE ON MEDICAL EDUCATION, Palmer House, Chicago, Ill., U.S.A., Aug. 30–Sept. 4. For information address. Dr. Louis H. Bauer, 10 Columbus Circle, New York 19, N.Y., U.S.A.

A workhorse "mycin" for common infections

respiratory infections

prompt, high blood levels

consistently reliable and reproducible blood levels

minimal adverse reactions

With well-tolerated CYCLAMYCIN, you will find it possible to control many common infections rapidly and to do so with remarkable freedom from untoward reactions. CYCLAMYCIN is indicated in numerous bacterial invasions of the respiratory system—lobar pneumonia, bronchopneumonia, tracheitis, bronchitis, and other acute infections. It has been proved effective against a wide range of organisms, such as pneumococci, H. influenzae, streptococci, and many strains of staphylococci, including some resistant to other "mycins." Supplied as Capsules, 125 and 250 mg., vials of 36; Oral Suspension, 125 mg. per 5-cc. teaspoonful, bottles of 2 fl. oz.

new

CYCLAMYCIN®

Triacetyloleandomycin, Wyeth

Conforms to Code for Advertising

Wyeth®

Philadelphia 1, Pa.

stop diarrhea

solves acute diarrheal disease problems...

■ swiftly relieves symptoms ■ rapidly destroys bacterial pathogens (bactericidal rather than bacteriostatic) ■ succeeds where others fail against the enteric "problem pathogens" — increasingly prevalent, refractory strains of Staphylococcus, Escherichia, Salmonella and Shigella

...without creating new problems

■ does *not* upset the balance of normal intestinal flora
■ does *not* encourage monilial or staphylococcal overgrowth
■ does *not* induce significant bacterial resistance

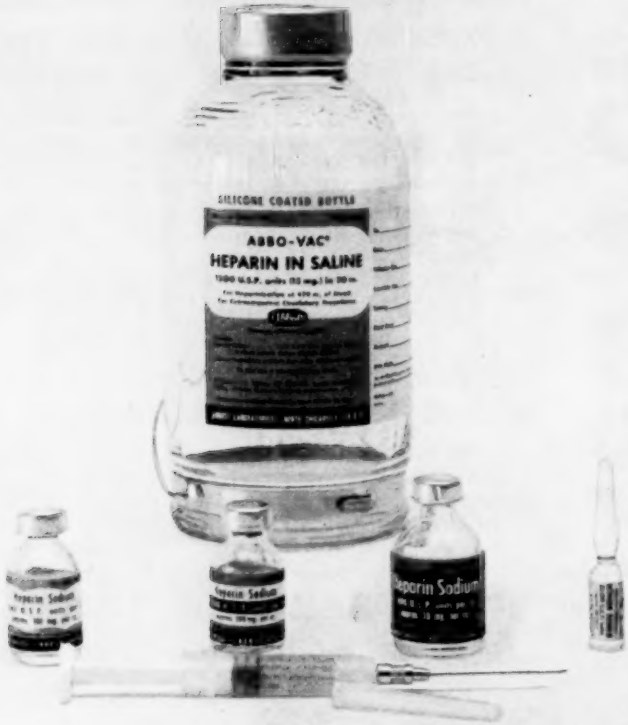
Furoxone® liquid

BRAND OF FURAZOLIDONE

A PLEASANT ORANGE-MINT FLAVORED SUSPENSION containing FUROXONE, 50 mg. per 15 cc., with kaolin and pectin ■ For patients of all ages (may be mixed with infant formulas, passes through a standard nursing nipple) ■ Dosage: Should provide (in 4 divided doses) 400 mg. daily for adults, 5 mg./Kg. daily for children ■ Supplied: bottles of 240 cc. (also: FUROXONE Tablets, 100 mg. scored, bottles of 20 and 100)

THE NITROFURANS—a unique class of antimicrobials
EATON LABORATORIES, NORWICH, NEW YORK

Some current pointers on heparin therapy



A helpful variety of anticoagulants is available to the medical profession. Among these, heparin has proved to be the most broadly useful. It is clearly the physician's agent of choice wherever he must reduce blood coagulability quickly.

Heparin cannot be effectively absorbed from the stomach or small intestine. It is administered only by injection. But the injection method chosen gives the physician considerable latitude.

Where speed of action is all-important, he will give aqueous heparin *intravenously*. Abbott supplies the concentrated solution in vials and ampoules. The physician may give intermittent intravenous injections of 5,000 to 10,000 U.S.P. units every four to six hours. Or, he may choose to dilute the solution for continuous intravenous infusion of about 40,000 units during 24 hours.

Where he plans long-term anticoagulant therapy, he may prefer to give the concentrated solution by *intramuscular* or *deep subcutaneous injection*. This technique greatly prolongs the action. Effect for as long as 12 hours is achieved by a single injection of 12,000 to 20,000 units. Adequate prolongation of clotting time is produced promptly—usually within 30 minutes to one hour.

This deep injection is accomplished most conveniently with the new Abboject® Disposable Syringe, containing 20,000 units in 1 cc. solution. Its heparin is not chemically combined with other agents, nor is it dispersed in a non-aqueous medium. Its extended action is quite comparable to that of non-aqueous repository forms, and provides worthwhile economy besides.

Heparin is well established as a help to the physician in treating thrombosis and embolism. It has improved his management of coronary occlusion, thrombophlebitis following surgery and childbirth, pulmonary embolism, retinal vascular disease, cerebral thrombosis, and even frostbite (essentially a thrombotic process).

It has also been useful in blood transfusions, cardiac surgery, and blood vessel surgery. With proper heparinization, blood flows across suture lines in arteries and veins, can be led through tubes, and traverses blood vessels with damaged intimal coats—without forming thrombi.

As another recent development, heparin has shown an ability to clear excess fats from the blood in atherosclerosis, angina, etc. This antilipemic effect seems independent of its anticoagulant activity.

Would you like our literature? Just write Professional Services, Abbott Laboratories, North Chicago, Ill.

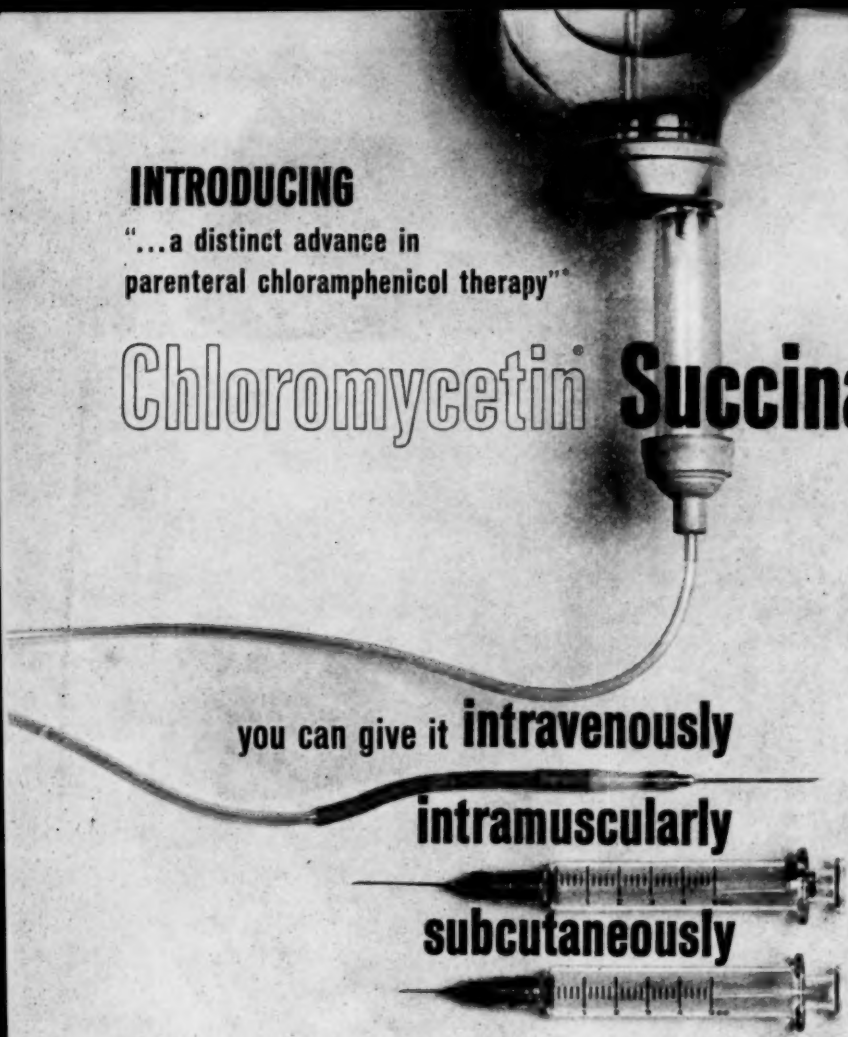
Abbott **HEPARIN**

(Heparin Sodium Solution, Abbott)

INTRODUCING

"...a distinct advance in parenteral chloramphenicol therapy"*

Chloromycetin® Succinate



you can give it intravenously

intramuscularly

subcutaneously

Highly soluble in water or other aqueous parenteral fluids, CHLOROMYCETIN SUCCINATE solution is easily prepared for use by recommended parenteral routes in a wide range of concentrations. Tissue reaction at the site of injection is minimal, permitting continuous daily dosage, even in children.

EXCELLENT CLINICAL RESULTS—CHLOROMYCETIN SUCCINATE provides broad-spectrum antimicrobial effectiveness and may be used whenever CHLOROMYCETIN is indicated. Since effective blood and tissue concentrations of the antibiotic are produced within a short time, clinical response is generally rapid. Signs of irritation at injection sites have been few.

SUPPLY—CHLOROMYCETIN SUCCINATE (chloramphenicol sodium succinate, Parke-Davis) is supplied in Steri-Vials,® each containing the equivalent of 1 Gm. of chloramphenicol; packages of 10.

CHLOROMYCETIN is a potent therapeutic agent and, because certain blood dyscrasias have been associated with its administration, it should not be used indiscriminately, or for minor infections. Furthermore, as with certain other drugs, adequate blood studies should be made when the patient requires prolonged or intermittent therapy.

*Rosa, S.; Puig, J. R., & Zaremba, E. A., in Welch, H., & Marti-Ibañez, F.: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, p. 817.

PARKE, DAVIS & COMPANY • DETROIT 32, MICHIGAN

66039

The Journal of MEDICAL EDUCATION

VOLUME 34 • NUMBER 4 • APRIL, 1959

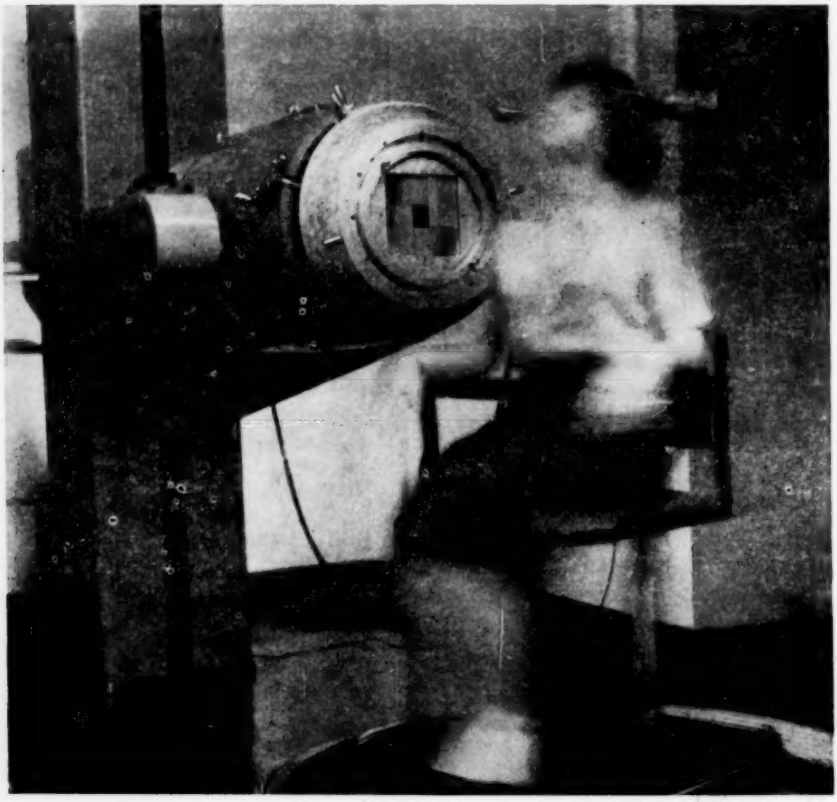
Table of Contents

While the patient whirls...

This represents treatment of a deep-seated neoplasm with a Cobalt-60 Beam Therapy Unit at Montefiore Hospital, New York.

Produced in large quantities in atomic energy installations, Co^{60} is being widely used as a source of radiation in teletherapy. It is less expensive than comparable supervoltage roentgen equipment and costs only $2 a curie compared with $20,000 a curie for radium.

While teletherapy with Co^{60} has certain technical and clinical advantages, radiotherapists stress an inherent limiting factor common to all megavoltage beam therapy—the reaction in normal tissues surrounding the tumor when tumoricidal quantities of external radiation are delivered. Internal administration of an isotope for effective localized radiation might avoid this hazard. In research circles, great hope is expressed that eventually newly synthesized compounds with marked affinities for specific tissues may be tagged with suitable radioisotopes for selective, localized treatment. Lakeside Laboratories, Milwaukee 1, Wisconsin.



34059

therapeutic direction through basic research LAKESIDE

A SYMPOSIUM ON GENETICS IN MEDICAL RESEARCH

University of Wisconsin, Madison, Wisconsin, April 7–10, 1958

Introduction

The development of Medical Genetics has lagged behind animal and plant genetics, and bacterial genetics. The most rapid growth has occurred during the past 10 years, and some formal teaching has been developed in the curricula of a few medical schools during this period. In 1953, a questionnaire revealed that 55 per cent of the Medical Schools in the United States and Canada gave some instruction in Genetics, but only six offered formal courses.

Research in medical genetics has shown a comparable rapid growth during the past ten years. Genetic implications in the causation and prevention of congenital malformations, metabolic diseases and degenerative phenomena represent fields for continued investigation.

This Symposium on Genetics in Medical Research was initiated from conversations between representatives of the N.I.H. and Wisconsin. The objective was the further expansion of genetics in medical research and education. An effort was made to show that experimental study on various laboratory organisms (animal, bacteria, and other) has the same role in medical genetics as the counterpart does in medical biochemistry and physiology.

Each Medical School in the United States was invited to send a teacher concerned with the development of a medical genetics program in that institution. Distinguished geneticists from the United States and other countries presented discussions around carefully selected topic areas. These principal discussions were followed by small group conferences with a maximum emphasis on individual participation.

The publication of these papers is a departure from the customary program of this Journal. However, because of their great potential significance in medical education (and the fact that the Editor was Chairman of the Planning Committee), it was felt that they should be published here.

JOHN Z. BOWERS, M.D.
Editor-in-Chief

Opportunities for Research in Human Genetics

JAMES V. NEEL, M.D., PH.D.
Department of Human Genetics, University of Michigan Medical School,
Ann Arbor, Mich.

From the position of this paper on the program, as well as the very pleasant postprandial state in which we now find ourselves, I would presume—and have presumed—that my presentation this evening was not intended to be characterized by closely measured logic applied to a very specific problem, but to be more in the nature of a general and perhaps somewhat rambling discourse.

Before getting on to the business at hand, I may, perhaps, be permitted to extend a few words of congratulation to our Wisconsin hosts, not only for the excellence of the program they have arranged, but for all the very significant recent developments here in the field of medical genetics. It is particularly noteworthy that this effort should materialize on a campus so long known for outstanding research in experimental genetics. As a relative "old timer" in the field of human genetics who has taken it upon himself to speak for the other old timers, I want to tell you, in all honesty and sincerity, just how heart-warming it is to see the quickening tempo of emergence, all over the country, of efforts like this one. There are those in this audience—I see Madge Macklin, Herluf Strandskov, and Franz Kallmann, to mention only three—who can remember even better than I the parlous state of human genetics a scant 15 or 20 years ago. The change in the intellectual—and financial—climate is truly staggering. I well recall how, at the time I abandoned Drosophila in favor of man as an object of genetic research, it was considered by many of my friends and associates as a very rash move, on the grounds that not only was it out of the question to do anything significant with human material, but there were just no positions to support the few wishing to work in the field. Today the shortage is clearly of men, not positions, while the subject matter of this Symposium alone seems adequate testimony on the question of significance.

By way of an introduction to my topic, I should point out that there are certain intellectual adjustments that the worker in the field of human genetics must make, especially if he enters the field from the background of experimental plant and animal genetics. He is now working with a material which not only cannot be bred at will but often must be pursued considerable distances, in order to determine the results of such breeding experiments as it may inadvertently have carried out. Even when there is the complete cooperation of the "material," there is this matter of generation time, which puts man in a very select class indeed. In this connection, there is one aspect of the development here at Wisconsin which I cannot resist commenting on. You have here a Department of Medical Genetics, headed by a microbiologist. This is quite different from our own development at Michigan, where we have a Department of Human Genetics, with the work on microbial genetics in the Department of Bacteriology. For myself, I'm just as happy to see it there. Having finally adjusted to the peculiar problems of human genetics, I must confess to still feeling a nostalgic and even envious twinge when I read or listen to a paper concerned with the fantastic manipulations of

the genetic material now possible in some of the micro-organisms. I can stand this twinge occasionally but fear that, were I working in really close proximity to someone like Dr. Lederberg, with day-to-day bulletins—any one of which covered almost as many generations as the whole of recorded human history—the twinges might come so frequently as to leave me in a state of status anginosus. Seriously, I think this close juxtaposition of work on the agent of disease as well as the host is really quite exciting, and a development to be watched with interest.

However, while the study of human genetics has its problems, it also has its advantages. There is an increasing number of questions which can be pursued just as effectively in man as in any other organism; and there is a growing list of questions which can be pursued even *more* effectively in man than in any other mammal. The grand strategy of human genetics lies in recognizing the advantages of man and making the most of them. Tonight I would like to touch upon some of the areas where human genetics can advance not only very rapidly, but with results which should illuminate the genetics of other organisms. If at places this sounds a little like a "pep" talk, then it is no coincidence, because this is what it is meant to be. Without for one moment minimizing all the practical problems with which the student of human genetics is confronted, I should like to emphasize, by means of a series of examples, the manner in which recent developments in medicine, demography, public health, etc., have improved the position of the human geneticist vis-à-vis his "experimental" organism. In point of fact, much of what we are discussing during this Symposium is eloquent testimony to these developments. The particular areas to come under discussion this evening include selected problems in biochemical genetics, in the genetic structure of populations, and in the nature of natural selection. Out of the many examples that could be chosen, I have, on the one hand, deliberately stayed away from areas to be covered by other speakers and, on the other hand, elected to draw rather heavily on some developments with which the group at Michigan has had first-hand experience, simply because this is the material with which I am most familiar. Other pertinent examples of the expanding horizons of human genetics, from the biochemical to the clinical level, will be found in such recent monographs as those of Harris (15) and McKusick (28).

THE STUDY OF NATURAL SELECTION IN MAN

Let us consider first the study of natural selection in man. In the final analysis, natural selection is the result of either differential mortality or differential fertility involving individuals of dissimilar genetic constitutions. That the human species may currently be subject to very significant selective pressures can scarcely be doubted—cf. Fisher (13); Neel (32); Crow (9). Many opportunities exist for the study of these pressures. In an experimental organism, such as the fruit fly, it is a very simple matter to introduce known numbers of individuals of differing genotypes into carefully controlled environments and to determine, after a given number of generations, the proportions in which the original and various derived genotypes are found. From this one can generate very precise statements of the relative reproduction of the different genotypes under these conditions, i.e., the selective value of the genotypes. One can go further, again using Drosophila as an example, and determine at what stage in the life cycle selection occurs, and, to some extent, how this selection takes place. However, many of the same types of observations can all be made in man, with a degree of detail which at least in part offsets the longer generation time. Thus, we certainly are in a better position to analyze the workings of both genetically determined differential mortality and fertility in man than in any other organism, complicated though the latter may be in man by psychic factors with which many organisms apparently do not contend.

Two recent examples will serve to illustrate how one can analyze in man the dynamics of the fertility difference between two genotypes. The first concerns multiple neurofibromatosis (Crowe, Schull, and Neel [10]). Individuals afflicted with this dominantly inherited trait have a reproductive expectancy at birth which is 52.7 per cent that of the normal siblings of sporadic cases of the disease. They are thus subject to rather severe negative selection. This impaired fertility can be analyzed into a number of component parts in a way which illustrates the interplay of physical and psychic components in determining an individual's fertility. To begin with, the disease results in a certain amount of disability and even mortality which clearly interfere with reproduction. For instance, in our series of 223 patients with the disease, there have been nineteen with fatal or incapacitating involvement of the brain or spinal cord prior to age 30, with the final proportion to be higher because many of the individuals in the series are still quite young. Life-expectancy curves for the disease are not available, but there can be no doubt that a diminished life span is a factor in the reduced reproductive expectancy.

There is no evidence that the disease is any more severe in one sex than the other. Nevertheless, there is a rather marked difference between the sexes in their relative fertility—that of males being 41.3 per cent the fertility of the male siblings of sporadic cases; and that of females, 74.8 per cent. In part, this is accounted for by a significantly lower marriage rate of affected males as compared with affected females. We have been unable to decide whether this implies greater discrimination on the part of the female of the species, or a greater social conscience on the part of the male, but at any rate, since the physical effects of the disease appear to be the same in the two sexes, this would seem to be more a sociological than a biological phenomenon. Even after marriage there are fertility differences, however, the married males showing 62.0 per cent of the fertility of the male siblings of sporadic cases, while the females exhibit 88.7 per cent the fertility of these female siblings. To what extent this is biological, and to what extent sociological, would be difficult to say. We have some indications of a lowered sexual drive on the part of affected males, a phenomenon certainly amenable to precise investigation. If this is actually the case, it might also be a factor in the lower marriage rate of affected males. Even were the sex drives of both affected males and females reduced equally, this might be reflected disproportionately in the reproductive performance of the male, because of the more passive role of the female in these matters. At any rate, there is clear evidence for the action of selective factors on at least four levels, with the analysis of the problem still in its early stages.

A second example of how some of the complexities of natural selection can be analyzed in man issues from a recent study on Huntington's chorea, another dominantly inherited trait (T. Reed and Chandler [40], T. Reed and Neel [42]). Stimulated by a report of the high fertility of individuals with Huntington's chorea in a particular kindred (S. Reed and Palm [39]), several years ago we undertook a study embracing all the families in the state of Michigan in which the disease has ever been recorded as occurring. For all individuals heterozygous for the gene responsible for this disease who met certain necessary restrictions and who had completed reproduction, the relative fitness, defined as the ratio of the mean number of children born to these heterozygotes to the corresponding mean of their normal sibs, was found to be 1.03 ± 0.11, i.e., not different from unity.[1] However, a significant difference was observed between males and females, the value for male heterozygotes being $.82 \pm .11$ and for females $1.25 \pm .14$, although from the clinical standpoint the disease pursues essentially the same course in the two sexes.

[1] The reference to heterozygotes rather than choreics is necessitated by the fact that, in all calculations, allowance must be made for a small amount of non-penetrance.

As in the case of neurofibromatosis, there is evidence that this difference may be due to both a lower marriage rate on the part of male gene carriers and a lower rate of reproduction after marriage. Possibly we see some kind of a general relationship emerging here.

The data were collected in such a fashion as to permit comparison with a properly stratified sample of the female Michigan population as a whole. When this comparison was made, the relative fitness of heterozygotes was estimated to be, not unity, but 0.79 ± 0.12. In other words, there are indications, at about the 5 per cent level of significance, that the relative fitness of both heterozygotes and their normal sibs is lowered, presumably because of psychic factors. The inference is that knowledge of the family background (and possibly other factors) depresses the reproductive performance of both groups. The danger of using sib controls in problems of this nature is apparent.

Individuals with chorea often die or are institutionalized prior to the completion of a normal reproductive span. Several years ago, T. Reed and I proposed the use, in problems of this nature, of a statistic known as "relative reproductive span," defined as weighted relative survival to and through the reproductive period (T. Reed and Neel [41]). Equating date of first institutionalization to death, we find the relative reproductive span for Huntington's chorea to be 0.93. There is the possibility, then, that during their active reproductive lives individuals with Huntington's chorea, especially females, actually reproduce at a slightly greater rate than their normal sibs, but that both groups are below the average reproductive performance of the inhabitants of the state of Michigan. I might say that, among other points, this study has given us a very healthy respect for how extensive a body of data one must have in order to dissect fertility differentials. Finally, infant and childhood mortality is slightly but significantly higher in the children of female choreics than in the children of their normal sibs. We do not have a satisfactory population comparison, but this figure appears less subject to the workings of distorting (psychic) factors which weaken sib comparisons than the one for fertility. We thus have evidence for selection due to mortality differentials at two different stages in the life cycle, evidence for selective factors related to the sex of the affected individual, and, finally, some evidence for a component of selection which may be due to psychic factors. Panse (37) has presented data from Germany on Huntington's chorea which, although not analyzed in this fashion, are susceptible to substantially the same interpretation.

Neither of these cases is by any means completely worked out. The elucidation of many points of interest must await the collection of far more material. Nevertheless, we have here illustrations of how one can begin to unravel the complexities of selection in man. Incidentally, you cannot have failed to note, in view of the findings with respect to the inadequacy of sibling controls in Huntington's chorea, a possible weakness of the study on neurofibromatosis, in which sibling controls were used for fertility estimates, although only the siblings of sporadic cases were utilized, and we have some data to indicate that their fertility is very similar to that of Michigan residents as a whole.

THE INVESTIGATION OF THE GENETIC STRUCTURE OF POPULATIONS

We turn now to consider how we may study the genetic structure of human populations. There's no use pretending we can approach this question with the elegance of the geneticist who works with Drosophila. On the other hand, when we turn to the mammalia, it now begins to appear that we can learn as much from man as from any other species. A high proportion of any wild-trapped collection of mammals either quickly succumbs to disease or fails to breed in captivity. There thus repeatedly arise questions concerning the representativeness of results with many mammal species. Add to this the reluctance of persons with com-

mercial interests in such animals as dogs and cats to reveal the results of such untoward genetic experiments as may develop under their observation, and you have a striking contrast to the situation in man, scattered all over the world in a profusion of different environments, in many of which there is a downright competitive eagerness on the part of some of its members to record their genetic misfortunes.

Three examples of the opportunities which the human species offers for work in this area immediately come to mind. We will be considering a fourth example at some length tomorrow. An important question for any species is the amount of concealed "recessively inherited" variability in the population and the relation of the magnitude of this concealed component to the amount of inbreeding in the population, this latter question having an important bearing on the issue of just how recessive so-called recessive genes really are. The social customs of man vary from the discouragement of consanguineous unions encountered in many European countries and our own, through the tolerance of consanguineous marriage which exists in Japan today, to the very obvious encouragement of consanguinity among the Basuto of southern Africa, where at least one third of a sample of 569 marriages contracted in the Southern Sotho Ward of Basutoland involved consanguinity (Ashton [3]).[2] There is every indication that behind the current practices of these areas lie centuries of tradition, even though for all areas the trend in recent years has probably been toward less consanguinity. No one has as yet been sufficiently enterprising to take real advantage of the Basutoland situation, an oversight which, in view of the current tempo of activity in human genetics, will almost certainly be rectified within the next decade; but we do have some figures on inbreeding effects for Europe (Sutter and Tabah [49, 50]; Böök [5]), and Japan (Yanase [53]; Shiroyama [47]; Ichiba [19]; Morton [29], and Schull [45]). They reveal what to me is surprisingly little difference in the results of first-cousin marriage in the two regions. Obviously, a great deal more in the way of data is needed, collected with due regard for extraneous variables, as well as knowledge of inbreeding coefficients; but the opportunities are clearly there.

A second type of problem in population genetics with respect to which the human species appears to offer unusual possibilities has to do with the significance of congenital defects. Something like 3 per cent of all births result in a child with a rather severe defect apparent before the age of 1 year. It has been customary to regard these unfortunate children as for the most part the accidents resulting from environmental insult or random mutational events. Recently (Neel [34]), I have attempted to pull together a number of scattered leads which collectively point to the possibility that a significant proportion of these children may be the "phenodeviants" resulting from complex, adaptive genetic systems with labile thresholds of expression, of the type discussed in particular by Lerner (25) and Dobzhansky (12). If this hypothesis is correct, children with congenital defect are to some presently unspecifiable extent to be regarded not as nature's mistakes so much as the price human populations pay for certain genetically adaptive systems. The validity of this hypothesis remains to be seen. The point to be made in the present context, however, is that because of man's numbers and subdivisions, together with his increasing concern for recording accurately the outcome of pregnancy, out of the world-wide vital statistics machinery can issue—should issue, with a few improvements—data bearing on the validity of this hypothesis, of a type which for one reason or another would be very difficult to obtain for any other mammal. Lest I appear overenthusiastic, let me recognize that the detailed breeding experiments necessary to nail this hypothesis down cannot be carried

[2] The representativeness of Ashton's population sample seems debatable, but, even if considerable bias exists, the fact of a high degree of consanguinity seems inescapable.

on in man, but require an experimental organism. On the other hand, a great deal pertinent to evaluating the hypothesis can be learned from man. In particular, careful study of the outcome of so-called interracial marriages is called for.

A final illustration of the opportunities offered by man in the field of population genetics stems from recent findings in West Africa as regards the distribution of the abnormal human hemoglobins. Studies on the distribution of the abnormal hemoglobins in West Africa have led to the recognition of two of the most striking gene "clines" or diffusion gradients known for any species. One of these is with respect to the gene responsible for hemoglobin C, with a "high" of approximately 10 per cent in Northern Ghana, and a decreasing frequency as one goes west, north, or east (cf. Neel *et al.* [35]; Lehmann [24]; Allison [2]). It is difficult to escape the impression that the present situation is a most unstable one, with the result that we human geneticists have an opportunity to study the details whereby changes in gene frequency come about, in a way which can be paralleled in only a few other species. Essentially the same situation exists for the gene responsible for hemoglobin S. There is increasing evidence that its introduction to parts of West Africa is, in terms of generations, relatively recent. For instance, Livingstone (26) has just described the following unusual situation in Liberia: a northwest to southeast cline as regards the frequency of this gene, with gene frequencies of approximately 10 per cent in the Mende and Kissi, and essentially zero in the Kru, Krahn, and Webbo. The other limb of this cline, in the Ivory Coast, is under active investigation at present. Inasmuch as the tribes of southeastern Liberia present many paleo-negroid traits which imply a degree of isolation from the surrounding tribes, once again the impression is of a gene on the march. These two situations offer exciting opportunities to come to grips with many questions regarding the details of how one gene replaces another in whole or part in the process of human evolution.

HUMAN BIOCHEMICAL GENETICS

Of all the areas where man seems to have come into the picture as an object of genetic study, biochemical genetics is preeminent. The developments with respect to the human hemoglobins at the moment provide one of the better known documentations of this statement. Human hemoglobin consists of one major and two minor components, the former termed A and the latter A_2 and A_3. Since 1949, no less than eleven inherited variations of the major component of human hemoglobin have been recognized. Because of the rarity of some of these types, as well as their restricted geographical distribution, the task of elucidating the genetic relationships of the genes responsible for these hemoglobins goes slowly, since critical evidence can be derived only from families in which at least two of the responsible genes are segregating simultaneously. The results of studying such families to date can be summarized as follows: The genes responsible for hemoglobins S and C appear to be alleles (Ranney, Larson, and McCormack [38]). There is evidence, unfortunately based on only one family, and thus greatly in need of confirmation, that the gene responsible for hemoglobin G segregates independently from that responsible for hemoglobin S (Schwartz *et al.* [46]). This leads to the postulate that at least two genetic loci contribute to the synthesis of the hemoglobin molecule. Very recently, evidence has appeared suggesting that the genes responsible for hemoglobin S and a new abnormal hemoglobin component, tentatively termed Hopkins-2, are segregating independently (Smith and Torbert [48]). The relationship of the locus responsible for the latter to the locus responsible for hemoglobin G is unknown and, in view of the rarity of both these types, not likely to be determined in the near future. The genetic relationship of the thalassemia abnormality to the hemoglobin S (and, by inference, C) locus is somewhat confused. There is increasing evidence that the traits are not segregating independently (Neel [33], Ceppellini [7]). On the other hand, there are

several families in which the traits have behaved as if due to genes at separate loci (summary in Neel [33]). The findings can be met either by the postulate of linkage or of a genetic heterogeneity of the thalassemia abnormality. The latter possibility would suggest the existence of at least two thalassemia loci, one allelic or closely linked to the S-C locus, the other independent. In summary, then, the fragmentary evidence presently available suggests the existence of several different loci involved in the production of the major component of human hemoglobin. Furthermore, Kunkel *et al.* (23) have recently produced evidence for inherited variations in the minor, A_2 component. The desirability of a speedy clarification of the genetic relationships of the various hemoglobins is underlined by recent developments now to be described concerning their biochemistry.

A considerable amount of work on the nature of the biochemical differences among the various hemoglobins has recently culminated in the very elegant demonstration by Ingram (20) that hemoglobins A, S, and C differ by a single amino acid substitution. The fact that allelic genes control in such a precise manner differences in proteins provides a striking demonstration of the precision of genetic regulation of body structure, a precision long postulated but only now demonstrated. The inference, that the nature and position of each of the 300 amino acids in each of the symmetrical half-molecules comprising human hemoglobin are coded into the chromosomal structures, is obvious. The further inferences concerning that coding system, when we consider the complexity of the human organism, are really rather staggering. Each successive announcement of the structure of an abnormal hemoglobin, as these are worked out, will constitute an important contribution to our understanding of gene action. Our knowledge of the structure of the hemoglobin variants is destined to progress much more rapidly than our knowledge of the genetic relationships of these variants. Here is one of those frustrating situations in human genetics—were man an experimental organism the elucidation of the situation could proceed so much more quickly. The grapevine has it that a number of microbial geneticists are in an urgent quest for a comparable instance of the genetic control of protein structure in a micro-organism, since a precision of genetic analysis never attainable in man can be anticipated. I doubt whether they will ever come up with a protein so conveniently packaged and so available for repeated samplings as hemoglobin.

One final, recent development will serve to illustrate very nicely the ferment in which human biochemical genetics finds itself, and especially the unexpected quarters in which material of great genetic interest may arise. World War II sparked an intense search for new and better antimalarials. Pamaquin (plasmochin) had been recognized in the 1930's as an effective antimalarial (James [21], *et seq.*); systematic studies were now undertaken on this drug, as well as a variety of its analogues (bibliography in "Symposium on Malaria" [51]). While both pamaquin and a number of its analogues such as pentaquin, isopentaquin, and primaquine proved to be highly effective in the prophylaxis of malaria, their use was limited by the frequency of hemolytic reactions encountered following their administration, especially in Negro troops (cf. Hockwald, Arnold, Clayman, and Alving [16]). Since one of these analogues, primaquine, proved to be a highly superior antimalarial, a great deal of interest devolved upon the mechanism of this hemolytic reaction. In a series of classical papers emanating from the Army Malaria Research Unit at the University of Chicago, under the direction of Dr. Alf S. Alving (for bibliography cf. Carson *et al.* [6]), it was shown that the ultimate cause of this susceptibility very probably lies in a loss of activity in the erythrocytes of sensitive persons of the enzyme glucose-6-phosphate dehydrogenase, a loss which in most studies on this system has been measured by a very marked decline in the re-

duced glutathione content of the erythrocytes following incubation with acetyl phenylhydrazine. Such a situation could not long escape the attention of some genetically oriented investigator; the recent excellent paper of Childs and his collaborators makes it quite clear that the trait is inherited, the hypothesis most consistent with the data being that the trait (measured by reduced glutathione content rather than enzymatic deficiency) is due to a sex-linked gene of intermediate dominance (Childs *et al.* [8]).

For many years it has been recognized that a small proportion of persons consuming the fava bean, a staple food item in the Mediterranean basin, were subject to a severe hemolytic anemia, a reaction termed "favism." This susceptibility has been noted to exhibit a familial trend (Luisado [27]; Murano [31]; Alcobé [1]; Szeinberg, Sheba, Hirshorn, and Bodonyi [52]). Within the past year it has been shown that individuals who develop favism are apparently deficient in the same enzyme the absence of which is responsible for primaquine sensitivity (Sansone and Segni [43, 44]; Szeinberg *et al.* [52]; Zinkham, Lenhard, and Childs [55]).

Finally, now, to round this story out, there is evidence that the well documented hemolytic reactions occasionally encountered following the administration of sulfanilamide, acetanilide, or nitrofurantoin, or after the accidental ingestion of naphthalene, may all be ascribed to this same enzymatic deficiency (Dern, Beutler, and Alving [11]; Zinkham, and Childs [54]; Kimbro, Sachs, and Torbert [22]). We have here, then, a most instructive example of how the recognition of a genetically controlled enzyme deficiency may unify a diverse and superficially unrelated set of phenomena. How many more such systems remain to be discovered? Not the least fascinating aspect of the system under discussion is that the effect of the enzyme deficiency on individual fitness remains so largely unknown. Childs *et al.* (8) calculate the frequency of the responsible gene in the American Negro as 0.136, from which, because of the known Caucasian admixture, one would infer a gene frequency of approximately 0.20 in the African ancestors of these Negroes. From the known frequency of favism in the Mediterranean basin, comparable gene frequencies probably exist in other ethnic groups. Frequencies of this magnitude for a sex-linked gene in the face of the negative selection imposed by favism clearly imply some selective advantage of the trait, of which nothing is known at present.

A number of recent authors have pointed out the extent to which drug idiosyncrasies may be a means of uncovering previously unsuspected biochemical systems with important genetic implications (Neel and Schull [36]; Haldane [14]; Motulsky [30]). The current flowering of the pharmaceutical industry cannot fail to bring to light large numbers of biochemical differences between individuals. Motulsky (30) has recently enumerated some of the more promising leads for genetic investigation stemming from drug idiosyncrasies. To his list I would like to add the differences among individuals in their ability to acetylate the anti-tuberculous drug isonicotinic acid hydrazide (Hughes, Biehl, Jones, and Schmidt [18]) and the susceptibility of some persons to chlorpromazine jaundice (for the possibility that the latter phenomenon should be interpreted as an allergic reaction, see Hollister [17]). In what other animal species besides man is there such a wealth of biochemical material awaiting investigation?

In closing, just a word about one very practical aspect of research in human genetics—the finances. The geneticist interested in microorganisms or Drosophila can often obtain very significant results with both limited space and budget; the physician interested in medical research can take advantage of many normal medical procedures in the development of his research program; but the human geneticist, with respect to many of the problems that need studying, must seek out, for a variety of special studies, large numbers of persons who would not otherwise be examined. This requires both space and funds. I wonder if

up to this point we have permitted ourselves to think on the appropriate scale. If man's germ plasm is what distinguishes him from the other animals, and if the problems confronting that germ plasm today are as great as we think they are, then surely it is time for an entirely different magnitude of effort. However, this effort can gain momentum no more rapidly than the personnel situation permits, and here there is a critical shortage indeed. In this connection, let me call your attention to the fact that the recently inaugurated Research Training Grant Program of the U.S. Public Health Service specifically recognizes genetics as an acute shortage area. Here is a means for tremendously increasing our training potential. In view of current trends in human biology, there seems no reason to doubt a continuing strong demand for the services of such trainees.

Finally, it remains only to state my firm conviction that, during the last half of the twentieth century, unlike the first half, studies on man will contribute quite as much to our understanding of a wide variety of genetic problems as will studies on any other animal.

REFERENCES

1. ALCOBÉ, S. Estudio genetico del foco de fabismo de Menorca-Ponencia oficial: fabismo y hemolisis alimentaria. IV. Congreso International de Higiene y Medicina Mediterraneas-Barcelona, pp. 53–60, 1953.
2. ALLISON, A. C. The Sickle-cell and Haemoglobin C Genes in Some African Populations. Ann. Human Genet., **21**:67–89, 1956.
3. ASHTON, E. H. The Social Structure of the Southern Sotho Ward. Communications from the School of African Studies, N.S. No. 15, Univ. of Cape Town, pp. 34, 1946.
4. ASHTON, H. The Basuto, pp. xi and 355. London: Oxford University Press, 1952.
5. BÖÖK, J. A. Genetical Investigations in a North Swedish Population. Ann. Human Genet., **21**:191–221, 1957.
6. CARSON, P. E.; FLANAGAN, C. L.; ICKES, C. E.; and ALVING, A. S. Enzymatic Deficiency in Primaquine-sensitive Erythrocytes. Science, **124**:484–85, 1956.
7. CEPPELLINI, R. Studies on the Allelism of the Genes Responsible for Thalassemia and the Sickling Phenomenon (in manuscript).
8. CHILDS, B.; ZINKHAM, W.; BROWNE, E. A.; KIMBRO, E. L.; and TORBERT, J. V. A Genetic Study of a Defect in Glutathione Metabolism of the Erythrocytes. Bull. Johns Hopkins Hosp., **102**:21–37, 1958.
9. CROW, J. F. Some Possibilities for Measuring Selection Intensities in Man. Am. Anthrop., **60**:1–13, 1958.
10. CROWE, F. W.; SCHULL, W. J.; and NEEL, J. V. A Clinical, Pathological, and Genetic Study of Multiple Neurofibromatosis, pp. ix and 181. Springfield: C. C Thomas, 1956.
11. DERN, R. J.; BEUTLER, E.; and ALVING, A. S. The Hemolytic Effect of Primaquine. V. Primaquine Sensitivity as a Manifestation of a Multiple Drug Sensitivity. J. Lab. & Clin. Med., **45**:30–39, 1955.
12. DOBZHANSKY, T. A Review of Some Fundamental Concepts and Problems of Population Genetics. Cold Spring Harbor Symp. Quant. Biol., **20**:1–15, 1955.
13. FISHER, R. A. The Genetical Theory of Natural Selection, pp. xiv and 272. Oxford: The Clarendon Press, 1930.
14. HALDANE, J. B. S. The Biochemistry of Genetics, pp. 144. London: Allen & Unwin, Ltd., 1954.
15. HARRIS, H. An Introduction to Human Biochemical Genetics. Eugenics Laboratory Memoirs, **37**:iv, 96. London: Cambridge Univ. Press, 1953.
16. HOCKWALD, R. S.; ARNOLD, J.; CLAYMAN, C. B.; and ALVING, A. S. Toxicity of Primaquine in Negroes. J.A.M.A., **149**: 1568–70, 1952.
17. HOLLISTER, L. E. Allergy to Chlorpromazine Manifested by Jaundice. Am. J. Med., **23**:870–79, 1957.
18. HUGHES, H. B.; BIEHL, J. P.; JONES, A. P.; and SCHMIDT, L. H. Metabolism of Isoniazid in Man as Related to the Occurrence of Peripheral Neuritis. Am. Rev. Tuberculosis, **70**:266–73, 1954.
19. ICHIBA, M. Study on Children Born as a Result of Consanguineous Marriage. II. On Their Physical and Mental Constitutions. Nihon Ika Daigaku Zasshi, **21**:113–18, 1953.
20. INGRAM, V. M. Gene Mutations in Human Haemoglobin: the Chemical Difference between Normal and Sickle Cell Haemoglobin. Nature, **180**:326–28, 1957.

21. JAMES, S. P. Some General Results of a Study of Induced Malaria in England. Trans. Roy. Soc. Trop. Med. & Hyg., **25**: 477, 1931.
22. KIMBRO, E. L.; SACHS, M. V.; and TORBERT, J. V. Mechanism of the Hemolytic Anemia Induced by Nitrofurantoin (Furadantin). Bull. Johns Hopkins Hosp., **101**: 245–57, 1957.
23. KUNKEL, H.; CEPPELLINI, R.; DUNN, L. C.; and FIRSCHEIM, I. L. A Variant in the Minor Component of Hemoglobin. Fed. Proc. (in press).
24. LEHMANN, H. Distribution of Abnormal Haemoglobins. J. Clin. Path., **9**:180–81, 1956.
25. LERNER, I. M. Genetic Homeostasis, pp. vii and 134. New York: Wiley & Sons, 1954.
26. LIVINGSTONE, F. B. The Distribution of the Sickle Cell Gene in Liberia. Am. J. Human Genet., **10**:33–41, 1958.
27. LUISADO, A. Favism. Medicine, **20**:229–50, 1941.
28. MCKUSICK, V. A. Heritable Disorders of Connective Tissue, pp. 224. St. Louis: C. V. Mosby Co., 1956.
29. MORTON, N. E. Empirical Risks in Consangineous Marriage: Birth Weight, Gestation Time, and Measurements of Infants. Am. J. Human Genet., **10**:344–49, 1958.
30. MOTULSKY, A. G. Drug Reactions, Enzymes, and Biochemical Genetics. J.A.M.A., **165**:835–37, 1957.
31. MURANO, G. Contributo alla conoscenza della predisposizione allergica eredofamilare nel favismo. Pediatria, **59**:479–515, 1951.
32. NEEL, J. V. The Study of Natural Selection in Primitive and Civilized Human Populations. Am. Anthrop., **60**:43–72; Human Biol., **30**:43–72, 1958.
33. ———. Genetic Aspects of Abnormal Hemoglobins. Nat. Res. Council, Conference on Hemoglobin. J. Nat. Acad. Sc. Publ. 557, pp. 253–71, 1958.
34. ———. A study of Major Congenital Defects in Japanese Infants. Am. J. Human Genet., **10**:398–445, 1958.
35. NEEL, J. V.; HIERNAUX, J.; LINHARD, J.; ROBINSON, A. R.; ZUELZER, W. W.; and LIVINGSTONE, F. B. Data on the Occurrence of Hemoglobin C and Other Abnormal Hemoglobins in Some African Populations. Am. J. Human Genet., **8**:138–50, 1956.
36. NEEL, J. V., and SCHULL, W. J. Human Heredity, pp. 361. Chicago: University of Chicago Press, 1954.
37. PANSE, F. Die Erbchorea, eine klinisch-genetische Studie. Leipzig: Georg Thieme, 1942.
38. RANNEY, H. M.; LARSON, D. L.; and MCCORMACK, G. H., JR. Some Clinical, Biochemical, and Genetic Observations on Hemoglobin C. J. Clin. Investigation, **32**: 1277–84, 1953.
39. REED, S. C., and PALM, J. D. Social Fitness versus Reproductive Fitness. Science, **113**: 294–96, 1951.
40. REED, T. E., and CHANDLER, J. H. Huntington's Chorea in Michigan. I. Demography and Genetics. Am. J. Human Genet., **10**:201–25, 1958.
41. REED, T. E., and NEEL, J. V. A Genetic Study of Multiple Polyposis of the Colon (with an Appendix Deriving a Method of Estimating Relative Fitness). Am. J. Human Genet., **7**:236–63, 1955.
42. ———. Huntington's Chorea in Michigan. II. Selection and Mutation. *Ibid.* (in press).
43. SANSONE, G., and SEGNI, G. L'instabilita del glutatione ematico (GSH) nel favismo. Utilizzazione di un test selettivo. Introduzione al problemo genetico. Boll. d. Soc. Ital. di Biol. Sper., **33**:1057–60, 1957.
44. ———. Sensitivity to Broad Beans. Lancet, **2**:295, 1957.
45. SCHULL, W. J. Empirical Risks in Consanguineous Marriages: Sex Ratio, Malformation, and Viability. Am. J. Human Genet., **10**:294–343, 1958.
46. SCHWARTZ, H. C.; SPAET, T. H.; ZUELZER, W. W.; NEEL, J. V.; ROBINSON, A. R.; and KAUFMAN, S. F. Combinations of Hemoglobin G, Hemoglobin S, and Thalassemia Occurring in One Family. Blood, **12**:238–50, 1957.
47. SHIROYAMA, E. On the Relation of Growth of Newborn Infants, Sucklings, and Children to Consanguineous Marriage. (Kumamoto Daigaku) Taishitsu Igaku Kenkyûsho Hôkoku, **3**:462–64, 1953.
48. SMITH, E. W., and TORBERT, J. V. Study of Two Abnormal Hemoglobins with Evidence for a New Genetic Locus for Hemoglobin Formation. Bull. Johns Hopkins Hosp., **102**:38–45, 1958.
49. SUTTER, J., and TABAH, L. Effets de la

consanguinité de l'éndogemie. Population, **7**:249–66, 1952.

50. SUTTER, J., and TABAH, L. Structure de la mortalité dans les familles consanguines. *Ibid.*, **8**:511–26, 1953.
51. SYMPOSIUM ON MALARIA. J. Clin. Invest., **27** (Suppl.): 1–150, 1948.
52. SZEINBERG, A.; SHEBA, C.; HIRSHORN, N.; and BODONYI, E. Studies on Erythrocytes in Cases with Past History of Favism and Drug-induced Acute Hemolytic Anemia. Blood, **12**:603–13, 1957.
53. YANASE, T. Heredo-clinical Investigation Concerning the Highly Inbred Villages in Western Japan, Especially Shiba-mura and Miyazaki-ken. Igaku Kenkyu, **21**:183–209, 1951.
54. ZINKHAM, W. H., and CHILDS, B. The Effect of Vitamin K and Naphthalene Metabolites on Glutathione Metabolism of Erythrocytes from Normal Newborns and Patients with Naphthalene Hemolytic Anemia. Am. J. Dis. Child., **94**:420, 1957.
55. ZINKHAM, W. H.; LENHARD, R. E.; and CHILDS, B. A Deficiency of Glucose-6-Phosphate Dehydrogenase Activity in Red Cells from Patients with Favism. Bull. Johns Hopkins Hosp., **102**:169–75, 1958.

The Chromosomes of Man

CURT STERN
Department of Zoology, University of California, Berkeley, Calif.

Human chromosomes were first clearly pictured by Arnold in 1879 (Fig. 1*A*). His drawings from tumor cells show interesting details, including what obviously were multipolar spindles; but they are not suitable for even a rough estimate of the number and types of chromosomes. Flemming, in 1881/82 described and pictured mitoses from normal human tissue. Some twenty large and small chromosomes—V, J, and rod-shaped—are recognizable in a division figure of a corneal cell (Fig. 1*B*). In 1898, Flemming devoted a special note to the topic of the chromosome number in Man (12). He referred to his earlier drawings and stated that they intentionally included only the clearer structures seen. With improved optics Flemming restudied his single remaining slide and concluded, with cautious hesitation, that somatic human cells have certainly more than 22 and probably less than 28 chromosomes and that 24 seemed to be the true number. A succession of later investigators offered different opinions. Estimates or counts in number of chromosomes ranged from 8 to 40 and more with an inclination toward believing 24 to be the correct number.

It was not until the early 1920's that an agreement was even approached. In 1912, on the basis of a thorough study based on superior technique, Winiwarter reported 47 elements in the testes of human males and 48 in the ovaries of females. He concluded that Man belongs to the XX-XO type of sex determination and that the 47 chromosomes consisted of 23 pairs of autosomes and an unpaired X chromosome. Eleven years later Painter (1923) came to a similar view except for one point. He too counted 23 pairs of autosomes and an X chromosome. In addition, however, he saw a small partner to the X, a Y chromosome. Thus, according to Painter, the male, like the female, had 48 chromosomes. Other students (e.g., Minouchi and Ohta [35]) supported the conclusion that Man has 48 chromosomes, although Winiwarter and Oguma (cf. 61) on the basis of their very clearest figures kept on insisting that 47 is the number characteristic for the male. A few investigators reported more divergent numbers, but most of these accounts had obvious weaknesses. They did not change the opinion of the majority that the number of chromosomes in Man had been well established as being 48.

There are more important things to know about Man than the exact number of his chromatic elements. Chromosomes are assemblages of genes. Whether the genes are primarily discrete macromolecules or at least in part integrated into superunits within the chromosomes, evidence in several organisms clearly shows that growth and development can proceed normally on the basis of a multitude of chromosomal rearrangements within a species. There are many ways in which the genes may be packaged, and it would seem of minor consequence whether these packages number 30 or 40 or 50. Nevertheless, Man's chromosome number is at least as significant a biological constant as the number of his teeth, his fingers, or his neck vertebrae. Moreover, it is necessary to know his normal chromosome number if one wishes to determine whether abnormal numbers occur and whether causal relations exist between ab-

normal chromosomal content and abnormal function of cells and tissues.

It was perhaps from the latter angle that the question of the normal number of human chromosomes was reopened again. Following reports by others of variable numbers in different cells of several tissues and, particularly, in cancer cells, the erstwhile botanical cytologists, Tjio and Levan (54), prepared cultures of normal lung fibroblasts from four therapeutically aborted Swedish embryos. Within a few days after the initial explantation they prepared slides with numerous mitotic figures accumulated as the result of colchicine treatment. Tjio and Levan "were surprised to find that the chromosome number 46 predominated in the tissue cultures from all four embryos." Disregarding lower numbers of chromosomes in cells that seemed damaged, they found only four doubtful cases of 47 or 48 chromosomes. No less than 261 cells gave counts of exactly 46 chromosomes. Photomicrographs of two cells in metaphase which accompanied the account are the clearest pictures of the diploid human chromosome assembly yet presented (Fig. 2). While there was the possibility, invocable *ad hoc*, that lung tissue might contain fewer chromosomes than other tissues (particularly the ovaries and testes), Tjio and Levan concluded that it "would be the most natural explanation of (their) observations . . . to generalize (their) . . . present findings into (the) statement that the chromosome number of man is $2n = 46$." Thus, in 1956, 44 years after Winiwarter, and 33 years after Painter had "solved" the problem of the human chromosome number, the question was again raised.

It must seem strange to a non-cytologist and even to cytologists used to the chromosomes of grasshoppers, lilies, and indeed most multicellular and some unicellular organisms, that the normal number of human chromosomes became a matter of inquiry again as late as 1956. All of the means of settling this apparently simple question would have seemed to have been available for at least 60 years. No electron microscope was needed, no photospectroscopy, only some well fixed and stained preparations to be observed under the ordinary light microscope. It must be admitted that this, indeed, was the basically simple situation—but there are attenuating circumstances for the delay in arriving at a final answer. The chromosomes of warm-blooded animals are relatively numerous and crowded into a small nuclear volume. After the use of standard fixation technique they not only tend to lie on top of one another in the optical axis of the microscope but often to clump together (Fig. 7*A*). Even an experienced observer will frequently be unable to decide at many points whether he has one single bent chromosome before his eyes or two straight ones. Then, too, he would have to decide whether two well stained bodies, separated by a light area, are two separate whole chromosomes or only the two arms of a single one whose connecting kinetochore (centromere) had taken up the dye so weakly as to be indistinguishable from the background.

The way out of such ambiguities has been resolved by the use of techniques, partly new and partly old, but wrongly neglected. The *new* techniques involved the use of hypotonic solutions and colchicine pretreatment, resulting in a spreading apart and contraction of the chromosomes (Hughes [20], Hsu [17], Makino and Nishimura [32], Tjio and Levan [59], and Kodani [24]). This reduces the occurrence of optical overlaps of separate chromosomes (Fig. 7*B*). The *old* technique, the revival of which became important, was that of using unsectioned material. Whole cells from small pieces of tissue gently flattened or squashed, or from tissue cultures directly grown on cover glasses, gave better assurance than microtome-sectioned material that no element is lost or no element from another cell mistakenly included in a count. Moreover, in sectioned material the knife may have cut through a chromosome so that consecutive sections may have to be used to reconstruct the whole.

The challenge of Tjio and Levan's discovery of 46 chromosomes in human lung

tissue was soon met in England by Ford and Hamerton (1956) who analyzed the spermatogonia and spermatocytes of testicular biopsy specimens from three adult men. In all three 46 was the spermatogonial number, and 23 pairs were demonstrated most convincingly in the great majority of first spermatocytes (Fig. 4).

Had earlier investigators been mistaken in regarding 48 or at least 47 chromosomes as the correct number? Undoubtedly, error often breeds error. The number of 48, once apparently established, would easily acquire "official" status, and an investigator who would see 46 chromosomes in his slide might well be inclined either to reinterpret the figure as actually possessing 48, or to lay it aside as indecisive. Not even preparations made with the new techniques were immune from variations in interpretations. What seemed to be impressive microphotographs of cells with $2n = 48$ (Hsu [17]) are now held to be compatible with counts of 46 (Hsu [18]).

The power of the number 48 may also have taken hold of Painter (40) when he studied another primate, *Macaca mulatta* (*Rhesus macacus*). Having recognized 48 chromosomes in Man, Painter likewise saw 48 chromosomes in the monkey. Years later, Shiwago (48) in Russia, who could find only 42 chromosomes in the same species, attributed the divergence of results to possible strain differences. After almost 2 decades Darlington and Hague (9) and Chu and Giles (7), with modern techniques and tissue cultures, agree with Shiwago in the count of 42 chromosomes, and so do Rothfels and Siminovitch (1958) and Ford (unpublished). While the possibility of intraspecific variability in chromosome number remains, it appears at present that the old count of 48 for the rhesus monkey was off by six. It is only fair to add that Painter (39) also described the chromosomes of the "Brown Cebus" monkey (a species not identified more specifically) and felt free to report a number different from 48, namely, 54 chromosomes. The newest and at present the only acceptable determinations of chromosome numbers in primates other than Man, according to Darlington and Hague, Chu and Giles, and Bender and Mettler (1958) are 34 (2 species), 42 (6 species), 44 (1 species), 46 (1 species), 50 (1 species), 54 (3 species), 60 (2 species) and 66 (2 species).

Even while Ford and Hamerton (13) were presenting their findings of 46 chromosomes in the testes of Man at the first International Congress of Human Genetics in Copenhagen in August, 1956, Kodani was making ready to describe to the International Genetics Symposia in Tokyo "The caryotype of Man with the diploid chromosome number of 48" (22). Was there then new unresolved divergence of opinion? Soon after, Kodani (23) announced that he had evidence for "three diploid chromosome numbers of Man." Among Japanese, whose testes formed his material, he found some individuals in whom each clearly analyzable cell had 46 chromosomes (unpaired in spermatogonia and mostly paired in spermatocytes), other individuals in whom 48 was the constant number (Fig. 5), and one man whose testicular cells possessed 47 chromosomes. A new and unselected sample of fifteen Japanese (24) consisted of five men with 48, one with 47, and nine with 46 chromosomes. And, for the first time since Tjio and Levan's publication, a white male with 48 chromosomes is also reported by Kodani. Among eight testes from whites in Iowa one had 48 and seven 46 chromosomes.

At present, Kodani is the only cytologist who, since 1956, has found more than 46 chromosomes in normal tissues. Other investigators have encountered uniformly only individuals with 46 chromosomes as seen by Tjio and Levan. Altogether, omitting Kodani's data at the time of writing this review (February, 1958) the published reports giving a count of 46 chromosomes are based on nearly 60 individuals (Tjio and Levan [54], Ford and Hamerton [13], Bender [5], as well as unpublished findings of their own or others kindly communicated to me by Levan and Ford). Nevertheless,

not only Kodani's publications but also limited inspection of his slides at present incline me to accept his claims for the existence of polymorphism in the chromosome number of Man. Yet, more than a single interpretation can be applied even to clear figures, and the possibility of misinterpretation cannot be denied. In order to base one's judgment on general agreement one must ultimately wait and see whether the numbers 47 and 48 will also be reported by others than Kodani.

The nature of the chromosomal polymorphism.—Granted that different men have either 46, 47, or 48 chromosomes, what constitutes the intrinsic differences? Within various animal and plant species, naturally found differences in chromosome number are of two main types. One type consists in a differential distribution of constant chromosomal material. Differential distribution most frequently means that one two-armed chromosome found in some individuals may be represented by two essentially one-armed chromosomes in others. Recent well worked-out examples of this phenomenon which may affect one or more two-armed elements come, among others, from wild populations of grasshoppers (White [58]) and snails (Staiger [51]). Two mammalian species have also been found to contain individuals with widely divergent numbers of chromosomes. Specimens of the rodent *Gerbillus pyramidum* had 40, 52, and 66 chromosomes, but the total number of arms was nearly identical in all individuals, varying only from 74 to 78. It should be added that these different numbers characterize specimens, indistinguishable otherwise, but from different regions of Algeria and Israel. In addition, intrapopulational chromosomal polymorphism in the same species has also been noted in one locality, with 62 and 66 chromosomes, but the same number of chromosome arms was present in all animals (Wahrman and Zohavi [46]). The second mammalian species with chromosomal polymorphism is the common shrew, *Sorex araneus*. About fifty specimens invariably possessed 36 autosomal arms, but these were "assembled" in from 22 to 27 separate chromosomes (Sharman [47], Ford, Hamerton, and Sharman [17]).

Chromosomal polymorphism in Man does not follow the "Robertsonian principle" of a constant number of arms distributed variously among two-armed or one-armed chromosomes. According to Kodani (24), it belongs to a second type of phenomenon which accounts for differences in chromosome numbers. This is the possession by some individuals of chromosome material which is absent in others. "Supernumerary" chromosomes, which were first studied early in this century in plant bugs by E. B. Wilson, are now known to occur in many other insects, flatworms, and various plants, including maize and rye. In Man, Kodani finds that 46 chromosomes, recognizable by their sizes and shapes, are common to all individuals and that individuals with 47 and 48 chromosomes have, in addition to the 46 common elements, one or two extra chromosomes. The supernumerary chromosome is one of the smallest in the complement. It appears similar to the Y chromosome. In the spermatocytes of 47-chromosome individuals it remains unpaired, and in spermatocytes of 48-chromosome individuals the two supernumeraries usually pair. No multiple associations have ever been seen in a metaphase stage. This may be due either to lack of homology between the supernumerary chromosomes and any of the others, or to their shortness, which is correlated with the presence of usually only one chiasma or other type of junction per pair (see below).

As long as the behavior and possible multiple associations of the supernumerary chromosomes in the meiotic prophase are unknown, the origin and homology of this element will remain obscure. Its designation as a supernumerary chromosome implies that it is not an essential part of the human karyotype. The fundamental diploid constitution of Man may thus be said to consist of 46 chromosomes.

It is too early to discuss in detail the relative frequencies of individuals with and

without supernumerary chromosomes. The present limited data indicate that the 46 chromosome type is the most frequent and that, perhaps, the frequencies of 47 and 48 chromosome types are higher in Japanese than in European and American whites. Undoubtedly, the next few years will add considerable information to these topics, from various parts of the world.

One particular aspect already deserves a brief comment. It refers to the apparent paucity of 47 chromosome individuals. With random mating one would expect this group to be more frequent than the rarer of the two other chromosomal types. In reality, only a single individual with 47 chromosomes has yet been found in unselected material. The meaning of this deviation from expectation remains to be seen. It may be parallel to Müntzing's (37) finding on the supernumerary chromosomes of the grass, *Poa alpina*. In the pollen mother cells of different specimens the number of the supernumerary chromosomes ranged from two to eight, with even numbers more frequent than odd ones. As a rule, cells in the root tips do not possess any supernumerary chromosomes. Müntzing tentatively assumes that supernumerary chromosomes in early development have a tendency for nondisjunction. Accordingly, sister chromosomes move together to the same pole, one cell receiving the doubled number and the other none. It is further assumed that nondisjunction is directed in such a way that the future root cells are those which do not receive the supernumerary chromosomes at all, while the future germ cells get the doubled numbers. It is not impossible that such a mechanism may also be active in Man and that the finding of 46 chromosomes in somatic tissues is not an accurate index of the germinal situation. Divergence in chromosome number between somatic and germinal tissues is of regular occurrence in certain groups of insects. Here supernumerary chromosomes are regularly present in the germline, in low numbers or as high as about 80. In early stages of embryogeny they are eliminated from somatic cells so that extreme differences in chromosome number may be found between germ cells and any other type of cell (White [57], Beermann [4]). Whether a similar phenomenon exists in man will have to be resolved by study of somatic and germinal cells from the same individuals.

The karyotype.—Number alone is, of course, one of the least informative aspects of the chromosomes of a species. More specific information is provided by the karyotype, which includes not only numbers but also size, shape, and other individual characteristics of the chromosomes. These attributes are best seen in the more elongate mitotic chromosomes, but the pairs of more contracted meiotic chromosomes can also be used. Drawings and diagrams of the human karyotype prepared by several workers are reproduced in Chart 1. There is a considerable amount of general agreement as to the relative sizes of whole chromosomes and of their arms, but details vary. The longest chromosome is approximately five times the length of the shortest. Tjio and Levan distinguish 10 M chromosomes (median-submedian kinetochore; index, long arm: short arm, 1–1.9), 10 S chromosomes (subterminal kinetochore; index, 2–4.9), and 3 T chromosomes (nearly terminal kinetochore; index, 5 or more), while measurements of Hsu's and Kodani's drawings yield respectively: M, 13 and 16; S, 8 and 11; and T, 1 and 1. Kodani's karyotype includes the supernumerary chromosome, and so does perhaps Hsu's. Also, Kodani's diagram is based on meiotic first metaphase chromosomes instead of mitotic chromosomes and may for this reason give different proportions than those found by the other authors. At least in part the differences probably are due to different treatment of cells. Tjio and Levan emphasize that there is an element of arbitrariness involved in the distinction of M, S, and T chromosomes. Certainly, the present status of our knowledge of the human karyotype is not yet satisfactory.

Details concerning the structure of human chromosomes beyond size and form

have been derived from meiotic stages. The staining behavior of a short region in two autosomes (E and I in Kodani's karyotype diagram) and of all or nearly all of one arm of the X chromosome differs from that of all other chromosomal regions and fits the general characteristic of heterochromatin. Two chromosomes each bear a nucleolus in a specific region. One of these is chromosome L, studied first by Schultz and St. Lawrence (46). The other is a longer chromosome, the identity of which has not been established, since its nucleolus disappears before metaphase when the chromosomes can be defined for purposes of the karyotype diagram.

The pachytene stage of the meiotic prophase has lent itself to further study of chromosomal structure. The L chromosome associated with the larger of the two nucleoli has been described as having 22 chromomeres of various sizes and specific linear arrangement (Schultz and St. Lawrence [46], 1949, Fig. 6 A–B, Kodani [23]). General agreement with these findings has also been expressed by Yerganian (62), who employed a unique technique for the analysis of pachytene chromosomes (Fig. 6*c*). By subjecting fixed testicular tissue to the action of a Waring Blendor he was able to disrupt the cells

CHART 1.—The karyotype of man

I. From a spermatogonium; 46 autosomes plus X and Y (Painter [38]).
II. From a spermatogonium; 46 autosomes plus X (Winiwarter, after Painter [38]).
III. From a spermatogonium; 46 autosomes plus X and Y (Shiwago and Andres [49]).
IV. From a lung cell in tissue culture; treated with colchicine; 46 chromosomes arranged according to their length, in three groups characterized by medium-submedian, subterminal, and nearly terminal position of the kinetochore (Tjio and Levan [54]).
V. Diagrams for 23 autosomes plus X and Y from tissue culture of embryonic spleen (Hsu [17]).
VI. Diagrams for 23 autosomes plus X and Y from first spermatocyte divisions. The thin section in chromosomes E, I, and X signifies heterochromatin. Chromosome L bears a nucleolus (redrawn after Kodani [22]).

and by successive centrifugation of the suspended components of the disassociated tissues to obtain isolated paired pachytene chromosomes. Nine different types of such bivalents have been mapped in some detail (Fig. 6 *D–F*). They are characterized by their distinct forms, specific sequences of larger and smaller chromomeres or knobs, as well as other features. Such cytological mapping of chromosomes is likely to make use of artifacts induced by the treatment, but the relative constancy of their main features suggests that the artifacts are vitally preformed, depending on some type of differentiation of the chromosomes which is present in the living stage. It will, however, be necessary to complement these studies by the difficult analysis of chromosomes of whole nuclei in order to make more reliable the interpretation of isolated chromosomes and chromosome fragments.

The bivalent chromosomes of first meiotic metaphases are held together by what appear to be typical chiasmata (except for the XY pair which will be discussed separately). Ford and Hamerton have counted the chiasmata present at metaphase in a total of 23 cells and obtained a mean of about 56 chiasmata for the 23 chromosome pairs. The largest bivalent frequently has five chiasmata, and others may have as many as four. The smaller chromosomes often possess only a single chiasma per bivalent.

There has been a report that finer differences in chromosomal constitution may be observed among different men. Andres and Navashin (1) measured the relative lengths of six pairs of spermatogonial chromosomes in their own slides from two Russians and in a slide from one Japanese, loaned to them by Minouchi. They found no differences in three pairs but from 20 to 50 per cent greater lengths in the three largest chromosomes of the Japanese individual. Such differences perhaps depend on variations in degree of coiling of the chromosomes. Chromosome lengths are known to be gene-controlled in some plants. It is, however, not impossible that the differences observed by Andres and Navashin are consequences of non-genetic differences in the tissues they studied. Evans and Swezy (1928) measured the lengths of all chromosomes in ten mesenchyme cells from each of one male and one female, embryo and adult individual. They found chromosomes in the males to be considerably longer than those in the females, particularly so for the largest chromosomes. A similar sex relation was obtained for the chromosomes of two rat embryos, one male, one female. No other investigators have attempted to study this relation which at present can only be cited without further comment. (It can only be mentioned here that Shiwago [48] compared the morphology of the ten largest chromosomes of man with those of the rhesus monkey. There were some clear differences pair by pair, but on the whole a considerable degree of similarity. More extensive differences have been reported by Darlington and Hague [9].)

Other variations in chromosome characters have been suggested by findings of bridge-like connections between separating anaphase groups and of chromosome fragments in first divisions of spermatocytes of certain individuals (Koller [25], Slifer and Beams [50]; Fig. 3). These phenomena are typical for inversion heterozygotes, i.e., individuals in which the sequence of loci in one or two homologous chromosomes is inverted (e.g., 1-5-4-3-2-6-7, in relation to the other, 1-2-3-4-5-6-7). Apparent bridges and fragments can also be produced by adhesions of chromosome ends and laggard whole smaller chromosomes, and it is not known what the true cause of the unusual patterns of anaphases has been (Schultz and St. Lawrence). It is unlikely that inversions will not be found in Man, since they occur in many other organisms.

Kodani (24) has observed a single spermatocyte among fifteen of a 48-chromosome testes in which, in addition to the 24 pairs, there was a univalent extra chromosome, larger than the supernumerary. Probably, this extra chromosome owed its presence to nondisjunction. There exists a well docu-

mented case in the literature of a woman belonging to blood group AB, married to an O man, who bore a very deformed O child. It has been suggested that this child possibly came from an egg which, in consequence of nondisjunction, had received neither of the homologous chromosomes carrying the I^A or I^B blood group alleles (Levine, after Wiener [54, p. 185]). If this is indeed the explanation, then the presence of the abnormalities in the child could be hypothetically attributed to its possessing only a single chromosome of the relevant pair and this derived from the father. Levine's explanation may well be valid, but apart from mutation the recent discovery of a suppressor gene for blood group substance B (29) provides an alternative way of accounting for an apparent O child from an AB parent, although it would leave unexplained the presence of congenital malformations.

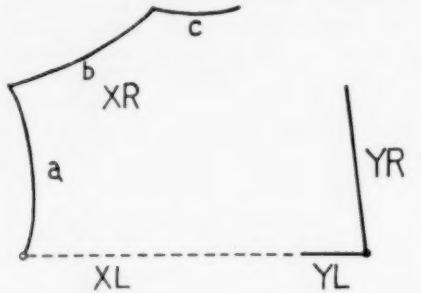


CHART 2.—Diagram of the paired X and Y chromosomes. *XR, XL; YR, YL:* right and left arms of the two chromosomes. *a, b, c,* the three segments of *XR*. The broken line for *XL* signifies heterochromatin (Kodani [22]).

The sex-chromosomes and sex-chromatin.—The nature and behavior of the sex chromosomes in Man have been a subject of much discussion. As stated earlier, Winiwarter, in 1912, on the basis of his counts of 47 chromosomes in testicular and 48 in ovarian tissues concluded that the male has a single unpaired sex chromosome, the X, and that the female has two X chromosomes. Painter (38, 39) observed both a relatively large X chromosome in the male and a small Y. His findings were upheld by various other cytologists, but Winiwarter supported by Oguma strongly maintained his original position (cf. 61). It is possible that their interpretation of cells as containing an XO type of sex-chromosomal constitution was partly based on 47-chromosome material in which the odd chromosome was a supernumerary one. However, the cytological methods available in earlier years made definitive conclusions difficult, and other cytologists (e.g., Minouchi and Ohta [35]) interpreted published figures with supposedly 47 chromosomes as actually showing 48 chromosomes.

There seems to be no doubt anymore that the human male, like most other mammals, possesses both an X and a Y chromosome (see the critical discussion by Matthey [33] and, among more recent authors, Sachs [43], Ford and Hamerton [13], and Kodani [23]). The two sex chromosomes form an unusually shaped bivalent in the first spermatocyte which makes it easily recognizable (note the remarkable similarity of this pair in some of the cells studied by Ford and Hamerton and by Kodani, Figs. 4 and 5). The X chromosome is of medium size, and the Y chromosome is one of the smallest of the complement. According to Kodani (23), the X chromosome consists of two arms, a long one in which three euchromatic sections are distinguishable by their being somewhat abruptly bent away from one another and a somewhat shorter heterochromatic arm which possibly terminates in a small euchromatic section (Chart 2). The Y chromosome consists of two euchromatic arms whose length are in the proportion of about 2:1. In bivalents the shorter arm is terminally joined to the tip of the short arm of the X chromosome. The nature of the meiotic association of the X and Y chromosomes has been the subject of much and sometimes sharp controversy. Matthey (34) has given a detailed analysis of the findings of himself and of others on the behavior of both human and non-human mammalian sex chromosomes. It now seems

likely that no true chiasmata are formed between the X and Y.

In 1952, Graham and Barr noted "a sex difference in the morphology of metabolic nuclei in somatic cells of the cat." The difference consists in the presence of a small, deeply stainable body in most nuclei of certain ganglion cells from females and the absence of this "sex-chromatin" in the majority of corresponding nuclei of males. Similar differences in sex-chromatin were soon found between human females and males in various tissues. The "Barr test" for sex-chromatin has become a powerful tool in the cytological analysis of human sex-deviants.

The discovery of sex-chromatin in mammalian nuclei was made without awareness of older work, but the phenomenon of persistent staining in non-dividing nuclei of parts of the sex-chromosomes which seems to be the basis of the sex-chromatin had long been analyzed in insect tissues. Sachs and Danon (45) have given particular attention to the cytological interpretation of what these authors call human "chromocenters" and to variations in their appearances in different tissues. In young spinous cells of the human skin they often find two separate, rather large, and equal-sized chromocenters in females but only a single such chromocenter plus a very small one in males. These they regard as of X and Y chromosomal origin, respectively. In a certain type of genetic intersexes, Danon and Sachs (8) find "that there is an excess of nuclei with three chromocenters in all their [i.e., the intersexes] skin cells." The authors do not make it clear whether they regard this as evidence of XXX or of XXY constitution, and one must look forward to further studies which may be of great importance for our understanding of the chromosomal determination of sex in Man.

The DNA content of human chromosomes.—As is well known, within a given species the amount of deoxyribonucleic acid (DNA) in the nuclei of diploid cells is usually constant. It is double that in the haploid gametes. In Man, Mirsky and Ris (36) found about 6×10^{-9} mg. of DNA in diploid nuclei.

Since the DNA is localized in the chromosomes and in all likelihood represents the material responsible for the transmission of genetic specificity, its quantity would seem to be a fundamental property of a cell. It is, therefore, at first surprising to learn that the DNA content of the sperm of a sample of 21 infertile men was found to be approximately one half of that of fertile men (26). No direct chromosome studies were made. DNA determinations of primary and secondary spermatocyte nuclei of the infertile males gave amounts four times and two times, respectively, greater than their sperm cells. This suggests that meiosis had proceeded as in fertile men, reducing the chromosomes from the four chromatid stages of the bivalents in the late meiotic prophase of the two chromatid stage of diakinesis and then to the single chromatid stage of the spermatid. If fertile men are characterized by DNA amounts in first and second spermatocytes and sperm of 4, 2, and 1 arbitrary units, the infertile on the average had 2, 1, and $\frac{1}{2}$ units, respectively. One may wonder whether each chromosome in the germ line of the sterile individuals is endowed with only one half of the DNA of that of chromosomes of fertile men. It would seem that chromosomes are composed of numerous identical chromosomal subunits. The cable-like multi-strand "polytene" chromosomes made famous by the salivary glands of many diptera would appear to be possible but not unique models for the composition of more ordinary chromosomes. The chromosomes of sterile men may then have fewer replicated subunits than those of fertile men.

A comparison between the DNA content of the nuclei of the mouse, the rat, and a variety of other mammals with that of Man gives very similar values for all of them (Mirsky and Ris [36], Vendrely [55]). The DNA content of Drosophila nuclei is only about one thirtieth of that of human nuclei. Since it seems unlikely that this means that man has 30 times as many

genes as the fly, the interpretation again is rather that the chromosomes of Man are composed of more equivalent subunits than those of the fly.

The chromosomes in somatic tissues and in tissue cultures.—This review cannot attempt a detailed discussion of the important work on chromosome numbers and karyotypes in *in vivo* and *in vitro* cultures of normal and tumorous human cells and on the related problems of constancy or variability of chromosomal numbers and types in somatic tissues (Beatty [3], Hsu [18]). The chromosome content of cultured cells has been shown to undergo numerous changes. It is presumed that, as a consequence of non-disjunction and a variety of aberrant division processes, cells with heteroploid, tetraploid, and higher multiple chromosome numbers originate (cf. Hsu and Moorhead [19]). On the other hand, multipolar mitoses followed by multiple cell divisions will lead to cells with subdiploid constitutions. Superimposed on these processes spontaneous chromosome breakages are observed with surprising frequency. They result in chromosomal reorganizations of various types such as translocations, formation of ring chromosomes, chromosomes with two kinetochores, and acentric chromosome fragments.

Not many years ago the majority of cytogeneticists would have assumed with confidence that most, if not all, changed karyotypes would exist only temporarily, since the normal balance of genes is absent. But this assumption and confidence has proved unjustified. The cells of most if not all known tumor strains have highly aberrant karyotypes, with the majority of strains characterized by cells with one of several hyperdiploid numbers and other strains consisting primarily of hypodiploid, hypotetraploid, or hyper-tetraploid cells (see Levan [27, 28], Ising and Levan [21]). Even strains with an identical or similar number of chromosomes may have very different karyotypes. In one strain specific chromosomes may all be present in equal numbers, while in another some may be present in excess and others under-represented. Or, rearrangements such as translocations may have distributed differently the chromosomal material over equal number of chromosomes. Often, within one strain there exists a variability in chromosome numbers, greater than is accountable by uncertainties in technique.

The main karyotype of each strain is perpetuated from cells which serve as a stemline. Other stemline cells, of different karyotypes, coexist in many strains and may become dominant with changes in the environment. Furthermore, reorganizations of karyotypes may occur so that the very concept of a constant stemline is only a relative one (cf., Makino [30, 31], Hsu [18]). Clearly, in tumor strains, as well as in strains derived from non-tumorous cells, new balances become established which under the conditions of culturing are selectively superior to those represented by the normal karyotype. These discoveries raise anew the question of the karyotype in somatic tissues within the intact individual. Formerly, the findings of abnormal chromosome numbers in somatic tissues, if technically acceptable, could well be thought of as of temporary significance only. Cells with such abnormal numbers were expected to have a short life in terms of either their own survival or that of their descendents. However, if cells in culture can be superior selectively by means of or in spite of abnormal karyotypes, there is less reason to insist that permanent tissues must be characterized by the karyotype found in the germline. The bearing of the cytological findings first made on cultures of mouse and rat tumor cells and now on cultures of human cells seems to go considerably beyond their significance for the cancer problem and to extend to the problems of normal differentiation. The old recognition that an understanding of normal growth and development is necessary for that of tumors finds its counterpart in the new insight into normal differentiation which the study of tumors may ultimately provide.

Chromosomes in human tissue cultures can be subjected to treatment such as radiation or exposure to chemical agents. Treatments of this kind have long been used within the intact body, particularly in con-

nection with the therapy of tumors. *In vitro* cultures—particularly if derived from single cells—make available for controlled experiments human cells and their chromosomes. Puck (42) has obtained survival curves for human cell populations after irradiation with x-rays which he interprets in terms of damage to two randomly distributed loci in the chromosomes. Bender (5) studied directly human chromosomes in tissue cultures after irradiation. In control cultures one or more chromosome fragments were observed in nearly 1 per cent of all cells. After irradiation with as little as 25 and 50 r units the percentages of fragments were 6 and 15, respectively. This high sensitivity of human chromosomes to irradiation is in line with expectation derived from animal experiments, but the data on human material obviously carry the highest weight in the evaluation of the effects of radiation on Man.

Human chromosomes as bearers of genes.—The orderly segregation and recombination of human genes, as observed by their consequences in successive generations and deduced from population-genetic considerations, place them in the chromosomes. In this, Man, of course, does not deviate from all other organisms which possess chromosomes. In detail, our knowledge of the chromosomal localization of genes in Man is still much more limited than that of many of the genetically more explored animals and plants.

At present only one single chromosome can be correlated with known genetic functions. This is the X chromosome, which has been identified since 1911 as the carrier of "sex-linked" (better designated as X-linked) traits (Morgan, Wilson). Many such X-linked genes have been found.

A number of published pedigrees seemed to show hologynic transmission of genes for several generations—from mothers to all of their daughters and none of their sons. These pedigrees had been tenatively interpreted in terms of X-linked genes with the peculiarity, known from strains of Drosophila, that the two X chromosomes of the human mother were assumed to be permanently attached to one another. A review of the best known human case of this kind, as well as the others, has removed much of the evidence for attached X chromosomes in Man (53).

The Y chromosome has been considered as the carrier of genes responsible for holandric transmission—from fathers to all of their sons and none of their daughters. Again, critical review failed to sustain sufficiently the claims of the Y chromosome for being the seat of any known genes (Stern [52], Penrose and Stern [41]). Not even the role of the Y chromosome in sex determination is understood. Nature's introduction of two instead of one variable in the genotypes responsible for the female-male difference, *viz.*, the 2X-1X *plus* the no Y-1Y alternative, has left the matter obscure. Does an XX conceptus develop into a female because it has two X chromosomes, because it has no Y, or because both facts play a role in its sexual determination? Is a man a man because he has only one X chromosome, or because he has a Y, or because of both? Perhaps the study of sex-chromatin in intersexes, after the manner initiated by Sachs and Danon, will bring us the answer soon. (*Addendum added in proof.* It has now been established, in Man and the mouse, that the Y chromosome determines maleness.)

For many years the X and Y chromosomes have been considered as probably carrying, in homologous segments, allelic genes which cross over, back and forth, from one sex chromosome to the other. The search for partial sex linkage has been fascinating and methodologically fruitful (Haldane [16] and later). But the case for existence of partial sex linkage has become so weak that no fully convincing example remains. Even the earlier cytological evidence for chiasmata between the X and Y chromosomes has been disputed and been declared as completely wanting (Sachs [44], Matthey [34]). Matthey has proclaimed the emancipation of the cytologist from the domination of the geneticist. On his part, the geneticist should be free to continue his probing for partial sex linkage independent of the findings of the cytologist.

Should this type of gene transmission ever be established, its physical basis will also be found.

The supernumerary chromosome of Man may perhaps deserve the negative distinction of being definable as free from specific genes. Its non-essential nature is obvious from its absence in individuals with 46 chromosomes. And the analogy with supernumerary chromosomes in other organisms suggests that it is made up of "non-specific" genic material—whatever this may actually mean.

None of the numerous autosomal genes in Man can be assigned to any particular one of the regular 22 pairs of autosomes. The future discovery of structurally variant homologous autosomes in different individuals and findings of correlations between their transmission and that of specific genes could lead to such assignments. Perhaps, even before this kind of knowledge is derived from work with individuals and populations, correlated cytological and genetic studies of cultured human cells will lead to localizations of genes in definite chromosomes.

Most human genes when followed simultaneously in transmission have been found to recombine independently. The presence of 23 chromosomal pairs makes the probability small of encountering two genes which are linked in the same chromosome. Furthermore, even genes located together in one chromosome but sufficiently far apart may be interchanged so frequently that they are virtually independent. Ford and Hamerton have called attention to the high frequency of chiasmata in Man and derived from it the expectation that crossing over between linked genes may have a very high frequency. This may be true, though to a limited extent only if the chiasmata—regarded as the sites of crossing over—would be found to be more or less restricted to limited regions in many chromosomes.

Obviously, all X-linked genes are located in the same chromosome, but attempts to map the X chromosome have been limited by the rareness in which heterozygosis for two X-linked genes is encountered in the same woman. (Simultaneous heterozygosis for three or more X-linked loci has never been seen yet.) Three 2-point linkages in the X chromosome have now been established, all of them involving the locus for red-green color blindness. One of the recombination values is of the order of 10 per cent, another about 25 per cent. The third is so close to 50 per cent that it is equivalent to free recombination and only recognizable as linkage by the X-linked transmission of the genes involved. The three two-point maps derived from these linkages cannot yet be combined into a unified four-point map.

Indications for autosomal linkages have been obtained in pioneer surveys by several investigators. Unequivocal evidence is still very limited, but three two-point linkages now seem well established with recombination values of between 3 and 10 per cent. They mark specific short stretches of chromosomes, but which chromosomes and which part of the chromosome the stretches represent is completely unknown.

The sum of the map lengths of all autosomes, estimated by Ford and Hamerton on the basis of chiasmatic frequency, is about 2700 crossover units. The sum of the three short stretches actually established is less than 1 per cent of this suspected total. More than 99 per cent of the genetic lengths of the human chromosomes remains to be mapped.

It would, however, be utterly misleading to end this survey of the human chromosomes in a pessimistic vein. We know so much more about the chromosomes of Man today than only a few years ago. We have abandoned misconceptions. We are working with new tools, with new ideas. Certainly, gaps will be filled and insights gained in steady progression.

ACKNOWLEDGMENTS

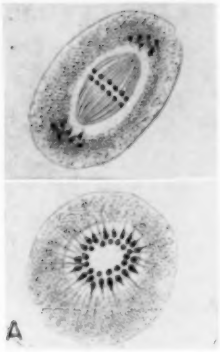
Grateful acknowledgment is made to Dr. Aloha Hannah-Alava for help in the preparation and critical review of the manuscript of this paper.

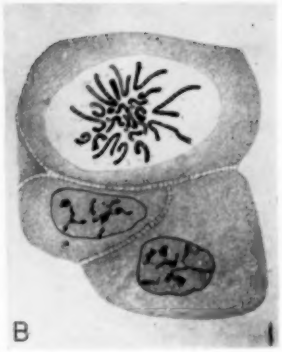
REFERENCES

1. ANDRES, A. H., and NAVASHIN, M. S. Morphological Analysis of Human Chromosomes. Proc. Maxim Gorky Med. Gen. Res. Inst. (Moscow), **4**:506–24, 1936.
2. ARNOLD, J. Beobachtungen über Kerntheilungen in den Zellen der Geschwülste. Virchow's Arch., **78**:279–301, 1879.
3. BEATTY, R. A. How Many Chromosomes in Mammalian Somatic Cells? Intern. Rev. Cytol., **3**:117–97, 1954.
4. BEERMANN, W., Nuclear Differentiation and Functional Morphology of Chromosomes. Cold Spring Har. Symp. Quant. Biol., **21**: 217–32, 1956.
5. BENDER, M. A. X-ray Induced Chromosome Aberrations in Normal Diploid Human Tissue Cultures. Science, **126**:974–75, 1957.
6. BENDER, M. A., and MITTLER, L. E. Chromosome Studies of Primates. Science, **128**:186–90, 1958.
7. CHU, E. H. Y., and GILES, N. H. A Study of Primate Chromosome Complements. Am. Nat., **91**:273–82, 1957.
8. DANON, M., and SACHS, L. Sex Chromosomes and Human Sexual Development. Lancet, **273**:20–25, 1957.
9. DARLINGTON, C. D., and HAGUE, A. Chromosomes of Monkeys and Men. Nature, **175**:32, 1955.
10. EVANS, H. M., and SWEZY, O. The Chromosomes in Man, Sex and Somatic. Mem. Univ. Calif., **9**:1–64, 1929.
11. FLEMMING, W. Beiträge zur Kenntniss der Zelle und ihrer Lebenserscheinungen. Part III. Archiv. f. Mikroskop. Anat., **20**:1–86, 1882.
12. ———. Ueber die Chromosomenzahl beim Menschen. Anat. Anz., **14**:171–74, 1898.
13. FORD, C. E., and HAMERTON, J. L. The Chromosomes of Man. Nature, **178**:1020–23, 1956.
14. FORD, C. E.; HAMERTON, J. L.; and SHARMAN, G. B. Chromosome Polymorphism in the Common Shrew. Nature, **180**:392–93, 1957.
15. GRAHAM, M. A., and BARR, M. L. A Sex Difference in the Morphology of Metabolic Nuclei in Somatic Cells of the Cat. Anat. Rec., **112**:709–23, 1952.
16. HALDANE, J. B. S. A Search for Incomplete Sex-Linkage in Man. Ann. Eugen., **7**:28–57, 1936.
17. HSU, T. C. Mammalian Chromosomes *in Vitro*. I. The Karyotype of Man. J. Hered., **43**:167–72, 1952.
18. ———. Numerical Variation of Chromosomes in Higher Animals. *Ibid.* (in press).
19. HSU, T. C., and MOORHEAD, P. S. Mammalian Chromosomes *in Vitro*. VII. Heteroploidy in Human Cell Strains. J. Nat. Cancer Inst., **18**:463–71, 1957.
20. HUGHES, A. Some Effects of Abnormal Tonicity on Dividing Cells in Chick Tissue Cultures. Quart. J. Micro. Sc., **93**:207–20, 1952.
21. ISING, U., and LEVAN, A. The Chromosomes of Two Highly Malignant Human Tumours. Acta Pathol. et Microbiol. Scandinav., **40**:13–24, 1957.
22. KODANI, M. The Caryotype of Man with the Diploid Chromosome Number of 48. Proc. Int. Genet. Symp. Cytol. Suppl., pp. 103–7, 1957.
23. ———. Three Diploid Chromosome Numbers of Man. Proc. Nat. Acad. Sc., **43**:285–92, 1957.
24. ———. The Supernumerary Chromosome of Man. Am. J. Human Genetics, **10**:125–40, 1958.
25. KOLLER, P. C. The Genetical and Mechanical Properties of Sex Chromosomes. III. Man. Proc. Roy. Soc. Edinb., **55**:194–216, 1937.
26. LEUCHTENBERGER, C.; WEIR, D. R.; SCHRADER, F.; and LEUCHTENBERGER, R. Decreased Amounts of Desoxyribose Nucleic Acid (DNA) in Male Germ Cells as a Possible Cause of Human Male Infertility. Acta Genetica et Stat. Medica, **6**:272–78, 1956.
27. LEVAN, A. Chromosome Studies on Some Human Tumors and Tissues of Normal Origin, Grown *in Vivo* and *in Vitro* at the Sloan-Kettering Institute. Cancer, **9**:648–63, 1956.
28. ———. Chromosomes in Cancer Tissue. Ann. N.Y. Acad. Sc., **63**:774–89, 1956.
29. LEVINE, P.; ROBINSON, E.; CELANO, M.; BRIGGS, O.; and FALKINBURG, L. Gene Interaction Resulting in Suppression of Blood Group Substance B. Blood, **10**:1100–1108, 1955.
30. MAKINO, S. The Concept of Stemline-Cells as Progenitors of a Neoplastic Population. Proc. Intern. Genetics Symp., 1956 Suppl. Vol. Cytologia: 177–81, 1957.
31. ———. The Chromosome Cytology of the Ascites Tumors of Rats, with Special Refer-

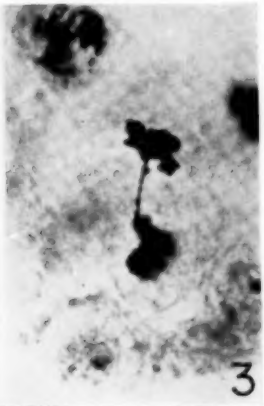
ence to the Concept of the Stemline Cell. *In:* G. H. BOURNE and J. F. DANIELLI (eds.), Intern. Rev. Cyt., **6**:25–84. New York: Academic Press, 1957.

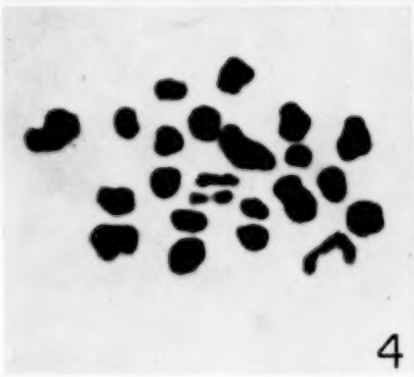
32. MAKINO, S., and NISHIMURA, I. Water-pretreatment Squash Technic. Stain Technol., **27**:1–7, 1952.
33. MATTHEY, R. Les Chromosomes des vertébrés. Lausanne: F. Rouge, 1949.
34. ———. Les bases cytologiques de l'hérédité "relativement" liée au sexe chez les mammifères. Experientia, **13**:341–46, 1957.
35. MINOUCHI, O., and OHTA, T. On the Number of Chromosomes and the Type of Sex Chromosomes in Man. Cytologia, **5**:472–90, 1934.
36. MIRSKY, A. E., and RIS, H. The Desoxyribonucleic Acid Content of Animal Cells and Its Evolutionary Significance. J. Gen. Physiol., **34**:451–62, 1951.
37. MÜNTZING, A. Accessory Chromosomes in *Poa alpina*. Heredity, **2**:49–61, 1948.
38. PAINTER, T. Studies in Mammalian Spermatogenesis. II. The Spermatogenesis of Man. J. Exper. Zool., **37**:291–321, 1923.
39. ———. The Sex Chromosomes of Man. Am. Nat., **58**:506–24, 1924.
40. ———. Studies in Mammalian Spermatogenesis. IV. The Sex Chromosomes of Monkeys. J. Exper. Zool., **39**:433–62, 1924.
41. PENROSE, L. S., and STERN, C. Reconsideration of the Lambert Pedigree (*Ichthyosis hystrix*). Ann. Human. Genetics, **22**:258–83, 1958.
42. PUCK, T. T. The Genetics of Somatic Mammalian Cells. Adv. Biol. & Med. Phys., **5**:75–101, 1957.
43. SACHS, L. Sex-linkage and the Sex-Chromosomes in Man. Ann. Eugen., **18**:255–61, 1954.
44. ———. The Possibilities of Crossing-over between the Sex Chromosomes of the House Mouse. Genetics, **27**:309–22, 1955.
45. SACHS, L. and DANON, M. The Diagnosis of the Sex Chromosomes in Human Tissues. Genetics, **28**:201–16, 1956.
46. SCHULTZ, J., and ST. LAWRENCE, P. A Cytological Basis for a Map of the Nucleolar Chromosome in Man. J. Hered., **40**:30–38, 1949.
47. SHARMAN, G. B. Chromosomes of the Common Shrew. Nature, **177**:941–42, 1956.
48. SHIWAGO, P. I. Recherches sur le caryotype du *Rhesus macacus*. Bull. Biol. et Med. Exper., **8**:3–13, 1939.
49. SHIWAGO, P. I., and ANDRES, A. H. Die Geschlechtschromosomen in der Spermatogenese des Menschen. Ztschr. Zellforsch, **16**:413–31, 1932.
50. SLIFER, E. H., and BEAMS, H. W. Chromatid Bridges and Fragments in Human Spermatocytes. Am. J. Human Genetics, **1**:79–82, 1949.
51. STAIGER, H. Der Chromosomendimorphismus beim Prosobranchier *Purpura lapillus* in Beziehung zur Ökologie der Art. Chromosoma, **6**:419–78, 1954.
52. STERN, C. The Problem of Complete Y-linkage in Man. Am. J. Human Genetics, **9**:147–66, 1957.
53. STERN, C., and WALLS, G. L. The Cunier Pedigree of "Color Blindness." Am. J. Human Genetics, **9**:249–73, 1957.
54. TJIO, J. H., and LEVAN, A. The Chromosome Number of Man. Hereditas, **42**:1–6, 1956.
55. VENDRELY, R. The Deoxyribonucleic Acid Content of the Nucleus. *In:* E. CHARGAFF and J. DAVIDSON (eds.), Nucleic Acids, **2**: 155–80, 1955.
56. WAHRMAN, J., and ZAHAVI, A. Cytological Contributions to the Phylogeny and Classification of the Rodent Genus *Gerbillus*. Nature, **175**:600, 1955.
57. WHITE, M. J. D. Cytological Studies on Gall Midges (Cecidomyidae). Univ. Texas Pub. **5007**:5–80, 1950.
58. ———. Adaptive Chromosomal Polymorphism in an Australian Grasshopper. Evolution, **10**:298–313, 1956.
59. WIENER, A. S. Blood Groups and Transfusion, 3d ed. Springfield, Ill: Charles C Thomas, 1943.
60. WINIWARTER, VON H. Études sur la spermatogenése humaine. I. Cellule de Sertoli. II. Hétérochromosome et mitoses de l'épthélium séminal. Arch. Biol., **27**:91–189, 1912.
61. WINIWARTER, DE H., and OGUMA, K. La Formule chromosomiale humaine. Arch. Biol., **40**:451–553, 1930.
62. YERGANIAN, G. Cytologic Maps of Some Isolated Human Pachytene Chromosomes. Am. J. Human Genetics, **9**:42–54, 1957.


A


B


2


3


4

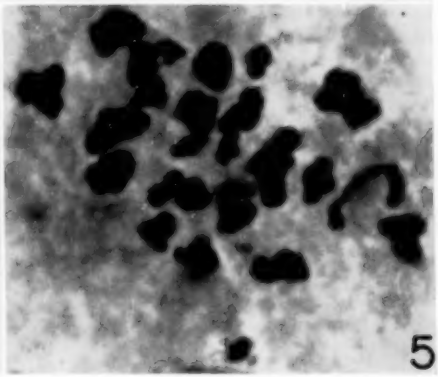

5

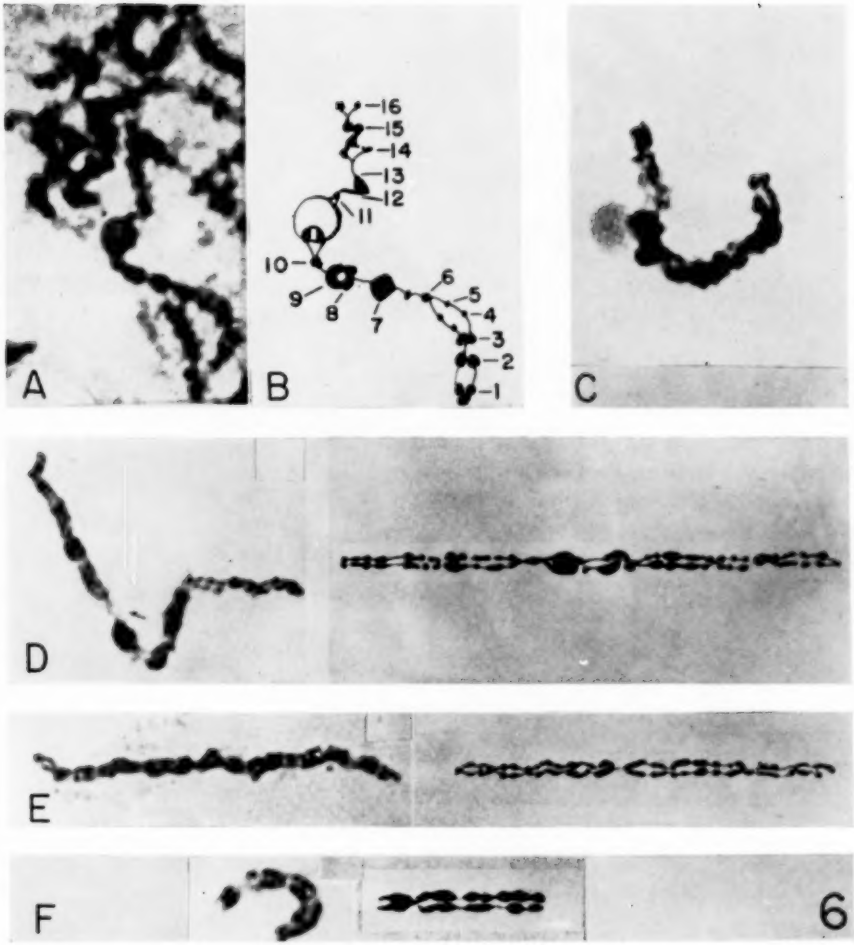
FIG. 1.—Early drawings of human chromosomes. (*A*) Dividing tumor cells (Arnold [2]). (*B*) Cells of the cornea (Flemming [11]).

FIG. 2.—Forty-six chromosomes in a cell of a tissue culture derived from an embryonic lung. Treatment with hypotonic solution has resulted in spreading apart and treatment with colchicine in contraction of the split chromosomes (Tjio and Levan [54]).

FIG. 3.—A chromosome bridge between the two anaphase groups of a first spermatocyte (Slifer and Beams [50]).

FIGS. 4 and 5.—Paired chromosomes in first meiotic divisions of human testicular tissue. Fig. 4: From an English male with 23 pairs of chromosomes (Ford and Hamerton [13]). Fig. 5: From a Japanese male with 24 pairs of chromosomes (Kodani [23]). Note the thin hooked pair of sex chromosomes in the extreme right area of each figure.

FIG. 6.—Pachytene chromosomes. (*A*, *B*) Photomicrograph and diagram (redrawn) of the chromosome with the larger of the two nucleoli (Schultz and St. Lawrence [46]. (*C*) Photomicrograph of the same (Yerganian [62]). (*D*, *E*, *F*) Photomicrographs and drawings of chromosomes I, II, and IX of Yerganian's series.



6

FIG. 7.—Models of the 46 chromosomes of man, in mitosis. *A*, according to formerly conventional techniques; *B*, according to modern techniques.

7A

7B

Methodology in Human Genetics*

ARTHUR G. STEINBERG
Department of Biology and Department of Preventive Medicine, Western Reserve University
Cleveland, Ohio

INTRODUCTION

The study of human genetics is complicated by our inability to control matings and by the fact that the length of a generation of the experimental organism is the same as that of the investigator. For these reasons and not because the genetics of man is basically different from that of other organisms, the human geneticist interested in problems other than gene action is required to use statistical methods of analysis, which geneticists working with other organisms need not employ.

Detailed analysis of hereditary patterns in man is limited for technical reasons almost entirely to characters determined by single gene differences. In general, the sequence of questions concerning the genetics of a character appears to be:

1. Is the character hereditary?
2. If so, what is the genetic mechanism?
3. How frequent is the gene in the population?
4. What is its mutation rate?
5. What are its linkage relations?

Another series of problems concerns genes in populations and populations of genes such as:

1. How do gene frequencies vary among populations?
2. How do they vary among groups (isolates) within a population?
3. What is the average mutation rate of a group of genes?
4. What is the average number of mutations carried by man?
5. What is the rate of response of human genes to various mutagens; in particular, to ionizing radiations?

Even if I were competent to do so, time would not permit me to review the methods of analysis used in these several problems. I have decided, therefore, to limit my discussion to some areas which the physician who is not a geneticist is most likely to want to use or to encounter. In the course of my presentation I shall provide references to more detailed discussions for those who wish further information.

David and Snyder (10) (p. 1130) asked, "What are the necessary and sufficient criteria for establishing that genetic constitution is significant in the etiology of a disease?" and answered (p. 1132), "An incidence of cases among the relatives of propositi significantly in excess of the incidence in the general population can be taken as substantial evidence that genetic factors are etiologically involved, *but only if it is shown that environmental factors cannot account for the increased incidence.*" (Italics provided.) David and Snyder are not the only ones to have posed and answered this question, nor were they the first. Indeed, most workers in human genetics have raised this question and answered it in the same way. I cite David and Snyder because of their excellent discussion of the difficulties which may be encountered in establishing this point.

DETERMINATION OF THE PATTERN OF INHERITANCE

If a significant increase in incidence, not due to environmental factors, has been found among the relatives of the probands,

* Supported in part by grants A-1822, A-1831, and H-3708 from the National Institutes of Health.

an attempt may be made to establish the genetic mechanism responsible for this increased incidence. As has already been stated, in practice only mechanisms involving single gene differences can be adequately analyzed in man. These differences may concern genes on the X chromosome or on some one of the autosomes. In either case, the mutant allele may be dominant, incompletely dominant, or recessive. In some instances patterns involving incomplete penetrance, situations in which those having the given genotype do not have a changed phenotype (see Snyder and David [37] for an excellent discussion), may be analyzed.

Autosomal dominant genes.—The simplest case is that of a rare, autosomal dominant gene with complete penetrance; here we expect one half of the parents, one half of the sibs, and one half of the offspring of the probands to be affected.

The expected values will be realized in large samples, provided we are careful to consider how the sample was selected. If the sample was selected via affected parents, 50 per cent of the offspring may be expected to be affected. What proportion may we expect to be affected if the families are selected via affected children? If we fail to take into account the way the families were selected, far more than 50 per cent of the offspring will be found to be affected.

As an example, consider the data reported by a nongeneticist, under the title, "Frequency of retinoblastoma in the progeny of parents who have survived the disease" (37). The data were derived from fifteen families, each with one parent who had survived the disease. There were 30 children in these fifteen families. Twenty-three of the 30 had had retinoblastoma, and only seven were healthy. This ratio is clearly closer to 3:1 than to the 1:1 expected. Fourteen of the fifteen families were ascertained because an affected child had been brought to the physician for treatment. It is clear that no family selected via an affected child can have fewer than one affected child. Hence, families of one child each will have 100 per cent of the children affected when only 50 per cent were expected. In this particular example the simple expedient of omitting the fourteen probands, through whom the families were detected, corrects this bias. When this is done the ratio of affected to normal offspring becomes 9:7, which is very close to the 8:8 expected. More elaborate methods of analysis are required for pedigrees in which more than one affected offspring was independently ascertained in different families. These will be discussed in connection with autosomal recessive inheritance.

Sex-linked dominant mutations.—Sex-linked dominant mutations may be readily recognized because all the sons of affected males are normal and all the daughters are affected, while on the average half the sons and half the daughters of affected females are affected. Thus, in a pedigree of a large kindred in which some members had hypophosphatemia, Winters *et al.* (46) found that none of the ten sons and all eleven of the daughters of affected males were affected, while six of fourteen sons and seven of thirteen daughters of affected females were affected.

Sex-linked recessive mutations.—A somewhat more difficult pattern to analyze correctly, at least to judge by the confusion in the literature, concerns sex-linked recessive characters. Here again, the pattern may be readily recognized. In the case of a rare, recessive sex-linked character, affected males have normal offspring and normal parents and are related to each other via females. Fifty per cent of the sons of all women with an affected father may be expected to be affected. Normal females who have had affected sons may expect half their daughters to transmit the character. Chart 1 (from Ref. 1) presents a pedigree characteristic of a sex-linked post-natal lethal character. The pedigree concerns an as yet unnamed disease characterized by thrombocytopenia, eczematoid dermatitis, bloody diarrhea, and draining ears (1, 17, 47). The pedigree certainly appears to be due to a sex-linked recessive gene lethal early in life. A common but incorrect pro-

cedure used to quantitate this impression is to count the number of affected and normal male offspring of females related to the first known heterozygous female via females. (Other more incorrect procedures are used, but we need not concern ourselves with those.) The error in this procedure lies in the assumption that all females in the pedigree, selected as previously indicated, are equally likely to have affected sons. A correct procedure is as follows:

Starting with the proband (VI-28, Chart 1), we find that his mother (V-16), grandmother (IV-6), and great-grandmother (III-5) all must be heterozygous for the gene, for it to have reached him. IV-7 must also be heterozygous, because she is an identical twin of IV-6; half the sons of these women may be expected to be affected. The probability that a daughter of any of these women is a heterozygote is $\frac{1}{2}$ and that such a daughter, if heterozygous, will have an affected son is again $\frac{1}{2}$; hence, the probability that grandsons, of III-5, IV-6, and IV-7, descended via daughters, would be affected is $(\frac{1}{2})(\frac{1}{2}) = \frac{1}{4}$. Similar reasoning gives the probabilities for each of the females in the pedigree. The result of such calculations is that 15.25 of

CHART 1.—Pedigree showing six generations of a family in which some male infants suffer from a sex-linked recessive disease (from Aldrich, Steinberg, and Campbell [1]).

the male infants in the pedigree were expected to be affected on the basis of a sex-linked recessive gene. Fifteen were observed to be so affected.

The incorrect procedure would have shown a considerable (but in this case not significant) difference between the observed and the expected values (fifteen affected and 25 normal observed, vs. 20 of each expected).

Autosomal recessive genes.—We turn now to the most difficult of the regular patterns to analyze, namely, the autosomal recessive. Li (20) has recently reviewed the methods appropriate to the analysis of pedigrees concerned with autosomal recessive inheritance. (Unfortunately, he has followed Fisher [13] and calls what is commonly known as the *a priori* or Apert method the proband method.)

The data for studies in which recessive inheritance is suspected usually consist of a series of families composed of parents and offspring. The families may have been found in any of several different ways. The method of analysis to be used is determined by the method of ascertaining (finding) the families. In general, families involving recessive characters are ascertained via affected children. Three different patterns of ascertaining the set of families may be recognized:

a) Every individual in a community may be examined regardless of his condition or that of his relatives;

b) Some affected individuals (one or more per family) may be found by direct observation, while others may be found because they are related to affected individuals; and

c) Each family may be selected via one and only one affected child regardless of how many children in the family may be affected.

Bailey (4) has called these "complete selection of affected individuals," "multiple incomplete selection of affected individuals," and "single selection of affected individuals," respectively, or, more briefly, "complete selection," "incomplete selection," and "single selection." More recently Morton (26) has suggested the terms truncate selection, multiple selection, and single selection, respectively, for those suggested by Bailey. Morton's suggested terms will be used in what follows.

The method of analysis to be used is determined by the method of ascertaining the families. Methods appropriate for each mode of ascertainment have been developed. They are listed in Table 1.

For truncate selection the maximum likelihood method is more efficient (has a smaller variance) than the *a priori* method, but the latter is considered easier to compute because tables of

$$\frac{sp}{1-q^s} \text{ and } \frac{spq}{1-q^s} - \frac{s^2p^2q^2}{(1-q^s)^2}$$

for $p = .25$ and $.50$ are readily available (16, 30). The maximum likelihood calculation is almost as simple if Finney's tables (12) are available.

The proband method is commonly used for samples obtained by multiple selection. It is not fully efficient (13), except in the limiting case of single selection (15). Bailey (3) has developed a maximum likelihood method for analyzing data collected by multiple selection, but it is laborious to compute. Methods appropriate to samples obtained by multiple selection may be used only when the number of ascertainments is known.

When ascertainment is via single selection, the proband method becomes identical with the maximum likelihood method and is the appropriate method to use. The estimate of p and its variance are not only easy to compute, but ascertainment via single selection may yield about twice as much information per index case as complete selection (9); hence, it seems that, when feasible, this is the method of choice. *It is important that the data be analyzed by the method suitable to the mode of ascertainment. Examples will illustrate this point as well as the methods.*

Examples of the effect of ascertainment on the method of calculation.—It should be

TABLE 1

SUMMARY OF METHODS FOR ANALYZING FAMILY DATA FOR AUTOSOMAL GENES

(Based on work of Weinberg [45], Fisher [13], Haldane [15], Bailey [4], and others.)

Ascertainment	Method of analysis	Estimate	Variance
Trunicate	*a priori*	$R_c = \sum_{s=2} \frac{sp}{1-q^s} n_s$	$\sum_{s=2} n_s \left[\frac{spq}{1-q^s} - \frac{s^2p^2q^2}{(1-q^s)^2} \right]$
	Maximum likelihood	$\frac{R}{p_c} = \sum_{s=2} \frac{sn_s}{1-q_c^s}$	$\frac{p_c q_c (1-q_c^s)^2}{\Sigma s (1-q_c^s - spq_c^{s-1}) n_s}$
	Using Finney's tables	$p_c = \frac{\Sigma WYn_s}{\Sigma Wn_s}$	$\frac{1}{\Sigma Wn_s}$

Where $\Sigma WYn_s = \frac{R}{pq} - \sum \left(\frac{s^2pq^{s-2}}{(1-q^s)^2} n_s \right) = \frac{R}{pq} - \Sigma Bn_s$

and $\Sigma Wn_s = \frac{\Sigma s (1-q^s - spq^{s-1})}{pq(1-q^s)^2} n_s.$

Multiple	Proband	$p_c = \frac{\sum_{r=1} \sum_{s=2} n_{sr} a (r-1)}{\sum_{r=1} \sum_{s=2} n_{sr} a (s-1)}$	$\sum_a \frac{1}{\Sigma a} \cdot \frac{pq}{(s-1)} \times [1 + p'_a + pp'_a (s-3)]$

where $p'_a = \frac{\sum_a \sum_r a (a-1) n_{sra}}{\sum_a \sum_r a (r-1) n_{sra}}$ = the probability of ascertainment.

Single (minimum)	Proband (maximum likelihood)	$p_c = \frac{R-N}{T-N}$	$\frac{(T-R)(R-N)}{(T-N)^3}$ or $\frac{p_c q_c}{T-N}$

a = number independently ascertained.
s = size of family.
r = number of affected in a family.
R = total number of affected in the sample.
n_s = number of families of size s.
n_{sr} = number of families of size s with r affected.
n_{sra} = number of families of size s with r affected of which a have been independently ascertained.
N = total number of families in the sample.
T = total number of children in the sample.
p_c = computed value of p.
$q = 1 - p$ = probability of not being affected.
p' = probability of being ascertained.
p = probability of being affected.

noted that in all analyses the family data must be grouped by type of mating as determined by the parents' phenotype. The matings must be grouped into those with both parents normal, those with one parent affected, and those with both parents affected. Matings of the first type are Aa × Aa (heterozygote by heterozygote) and have an expected frequency of affected offspring of .25, matings of the second type are Aa × aa with expected frequency of affected offspring equal to .50, while those of the third type are aa × aa with all offspring expected to be affected. In what follows we will consider only matings with both parents normal.

1. Truncate selection: When selection is truncate the families of different types will be represented in the sample in proportion to the frequency of its occurrence (see Li, 1954, for review and proof).

Table 2 presents a sample generated by assuming truncate ascertainment. Examples of the use of the *a priori* method are numerous and readily available (References 16, 31, 41, and others); therefore, I will not illustrate it. I will illustrate, instead, the maximum likelihood analysis using Finney's tables. This requires the selection of a preliminary value of p, the calculation of two values, and the division of one by the other. The values to be computed are shown in Tables 1 and 2. Finney (12) has published tables of $1/pq$, B, and W for a convenient series of values of p and for families of sizes two through 20. Since the data are being tested for recessive inheritance the most reasonable value of p to assume is .25. The details of the calculation are shown in Table 2.

The value for $1/pq$ with $p = .25, q = .75$, as read from Table 1 in Finney's paper (12) is 5.3333. This multiplied by 152 (the total number of affected in the sample) equals 810.662. Bn_s is obtained by multiplying the observed number of families of size s by the value of B appropriate to the value of s and the assumed value of p. The values of B are listed in Table 2 of Finney's paper. The products obtained over all values of s are summed to give ΣBn_s as shown in Table 2 of this paper.

The difference between R/pq and ΣBn_s equals ΣWYn_s. The latter divided by ΣWn_s provides the desired estimate of p. The variance of the estimate equals $1/\Sigma Wn_s$. ΣWn_s is found by reading from Finney's Table 2, values of W appropriate to s and the assumed value of p, multiplying this value of W by n_s and summing the products obtained for the different values of s. This calculation is also shown in Table 2.

The method is an iterative one, so the calculation should be repeated with the estimated value of p if it does not closely approximate the initially assumed value of p. In practice, sufficient accuracy is obtained by using a new assumed value of p which can be found in the tables. The value to be chosen is the next higher value of p to be found in the table if the originally assumed value of p is less than p_e and vice versa, the next lower value if the assumed value of p is greater than p_e. This will be illustrated in the next section.

Note that the use of a method appropriate to single selection greatly underestimates the value of p.

2. Single selection: In this case the probability of finding a family is directly proportional to the number of affected in the family. Therefore, the relative frequency with which a family of size s with r affected will be found is

$$r \frac{s!}{r!\,(s-r)!}\, p^r q^{s-r}.$$

This value, multiplied by the observed total number of families of size s, gives the expected number of families of size s with r affected.

The data in Table 3 were elaborated on the assumption that ascertainment was single and that all matings were Aa × Aa. The expected value of p is .25. The estimated value of p by the method appropriate to single ascertainment is of course also .25 (Table 3). The standard error is ±.04. The estimate of p by the maximum likelihood method appropriate to truncate as-

certainment with the aid of Finney's tables is also illustrated in Table 3.

The initial assumed value of p was the obvious one of .25. The estimated value of p (.401) was derived, as in the case of complete ascertainment, by Finney's tables for values of $1/pq$, B, and W, and computing the necessary products and sums. Since the estimated value differed greatly from the assumed value, the calculation was repeated using .40 for p. This gave a new estimate of p, .388. A third cycle was tried with the use of .35 for p, this being the next lower value of p in the

TABLE 2

A HYPOTHETICAL SAMPLE TO REPRESENT TRUNCATE ASCERTAINMENT OF A RECESSIVE CHARACTER

All matings were between heterozygotes

s	r	n_{sr}	n_s	t_s	R_s
2	1	42	49	98	56
2	2	7			
3	1	54	74	222	96
3	2	18			
3	3	2			
			123	320	152

Analysis: A.—Truncate ascertainment; maximum likelihood method. (All values in parentheses were read from tables in Finney, 1949.)

Assumed value of $p = .25$. $R = 152$

$$\frac{R}{pq} = 152\,(5.3333) = 810.662$$

$$-\Sigma Bn_s = -49\,(5.224) - 74\,(5.049) = -629.602$$

$$\Sigma WYn_s = \frac{R}{pq} - \Sigma Bn_s = 181.060$$

$$\Sigma Wn_s = 49\,(3.483) + 74\,(7.480) = 724.187$$

$$p_c = \frac{\Sigma WYn_s}{\Sigma Wn_s} = \frac{181.060}{724.187} = .2500$$

$$V = \frac{1}{\Sigma Wn_s} = .001381$$

$$\sigma = .037$$

B. Single ascertainment.

$$p_c = \frac{R-N}{T-N} = \frac{152-123}{320-123} = \frac{29}{197} = .147$$

$$V = \frac{(.147)\,(.853)}{197} = .000636$$

$$\sigma = .025 \qquad \frac{d}{\sigma} = \frac{.103}{.025} = 4.12$$

tables. The estimate of p was .389. Either .388 or .389 could be accepted; .388 was accepted. The value of $\Sigma W n_s$ appropriate to the estimation of the standard error of p (.388) is obtained by interpolation between the values of $\Sigma W n_s$ for $p = .35$ and $p = .40$. Note that the use of a method appropriate to complete selection has grossly overestimated p.

3. Multiple selection: Finally, for samples with known ascertainment which is neither complete nor minimum, Wienberg's proband method (13, 45) or Bailey's maximum likelihood method (3) may be used. Because such data are rarely available and the calculations to obtain a weighted mean estimate of p are extensive, I shall not detail them here. Those interested are referred to the original papers.

Haldane (15) has suggested that when ascertainment is unknown, p be estimated twice; once on the assumption of complete ascertainment (p_1) and once on the assumption of minimum ascertainment (p_0). The hypothesis of recessive inheritance is to be accepted when

$$p_1 + 2\sigma_{p_1} > .25 > p_0 - 2\sigma_{p_0} .$$

Since p_1 tends to overestimate the observed value of p if the data have not been collected by complete ascertainment, while

TABLE 3

A HYPOTHETICAL SAMPLE TO REPRESENT SINGLE ASCERTAINMENT OF FAMILIES WITH PARENTS HETEROZYGOUS FOR A RECESSIVE GENE

s	r	n_{sr}	n_s	R_s		t_s
1	1	30	30	30		30
2	1	36	48	36	60	72
2	2	12		24		24
3	1	27		27		81
3	2	18	48	36	72	54
3	3	3		9		9
	Totals:	126		162		270

Analysis: A.—Single ascertainment

$$p_c = \frac{R - N}{T - N} = \frac{162 - 126}{270 - 126} = \frac{36}{144} = .25$$

$$\sigma = \left(\frac{p_c q_c}{T - N}\right)^{1/2} = \left(\frac{(R - N)(T - R)}{(T - N)^3}\right)^{1/2} = \left(\frac{(.25)(.75)}{144}\right)^{1/2} = .0361 .$$

B.—Truncate ascertainment

1st cycle: $p = .25$ $R = 132$

$\Sigma W Y n_s = 210.892$

$\Sigma W n_s = 526.224$

$p_c = .401$

2nd cycle: $p = .40$ $R = 132$

$\Sigma W Y n_s = 193.844$

$\Sigma W n_s = 499.824$

$p_c = .388$

3rd cycle: $p = .35$ $R = 132$

$\Sigma W Y n_s = 191.947$

$\Sigma W n_s = 493.920$

$p_c = .389$

accept = .388

$\Sigma W n_s$ for .388 by interpolation = 498.407

$$\sigma_{p_c} = \left(\frac{1}{498.407}\right)^{1/2} = .045$$

p_0 tends to underestimate the observed value of p if the data have not been collected by minimum ascertainment, I have often wondered if Haldane's criterion is not too broad.

It seems to me to be highly unlikely that data collected by truncate ascertainment will not be known to have been so collected; consequently, p_1 will always overestimate p. Similarly, it is unlikely, but perhaps not as unlikely as in the case of truncate ascertainment, that data collected by single ascertainment will not be recognized to have been collected in this way; consequently p_0 will always underestimate p. For these reasons a more appropriate criterion may be

$$p_1 + \sigma_{p_1} > .25 > p_0 + \sigma_{p_0} .$$

This problem is being investigated further (Littell and Steinberg, unpublished).

Among the major limitations of the methods reviewed in the foregoing are that, in general, they are suitable only for alleles with essentially complete penetrance, which have a frequency considerably greater than the mutation rate and which determine a character for which there are relatively few phenocopies. Most characters of interest in human biology are not determined by genes which satisfy these criteria. Morton (23) has pointed out that the traditional statistical methods, "... fail to provide tests of internal consistency or to distinguish among alternative hypotheses which in principle are quite distinct, such as partial sex linkage and sex-biased manifestation, or mutations, phenocopies, and incomplete penetrance. Further progress in many aspects of human genetics must await the application, not only of better laboratory techniques, but also of a more refined methodology, made possible by modern developments in mathematical statistics."

Morton (24) is actively engaged in providing methods which promise to overcome some of the deficiencies of the older methods. These methods provide a test of the internal consistency of the data and an estimate of the frequency of phenocopies. For sex-linked genes they provide in addition a method for comparing the mutation rate in males with that in females.

In essence his procedure involves (*a*) the computing and comparison of three independent estimates of the probability of an affected individual being ascertained; this tests the independence of the ascertainments—the derived probability of ascertainment may then used in maximum likelihood methods to test the mode of inheritance; and (*b*) an estimate, based on the distribution of isolated and familial cases, of the proportion of the isolated cases which may be phenocopies. The method is designed to be applied to samples which have been obtained by multiple incomplete selection and *for which the enumeration of the individuals ascertained and the number of times each has been ascertained, are accurately known and reported.*

The development and extension of these methods offer an opportunity to study entirely new types of characters in man and to study former problems with much greater precision than was previously possible.

GENE FREQUENCY

Cotterman (7), in his fine summary of methods of estimating allele frequencies at a single locus, summarizes the uses of such estimates as follows:

1. comparing two or more populations, differing in geographical location, age composition, breed, sex, etc.;

2. testing homogeneity of a single sample, i.e., testing consistency of the genetic hypothesis and sampling theory in relation to the particular sample used in estimating the gene frequencies; and

3. analyzing other bodies of data, especially collections of family records, where the expectations in various offspring classes are functions of the gene frequencies.

To these we may add:

4. as a basic datum in the estimation of mutation rates and

5. in epidemiology, to estimate the po-

tential frequency of a disease with variable age at onset or incomplete penetrance.

The methodology of gene frequency estimates is enormous and cannot possibly be adequately discussed in a single session. My discussion will be more limited than that of Cotterman cited above. Those who wish further information are referred to his paper and to the excellent book by C. C. Li (21).

In what follows we will assume that the sample consists of randomly selected, unrelated (at least not closely related) individuals, all showing the same phenotype, which in turn is assumed to be due to an allele at one locus. We shall assume further that all estimates of gene frequency, with the exception of two allele systems without dominance such as the MN system, are based on the Hardy-Weinberg equilibrium (40) or some modification of it.

The Hardy-Weinberg equilibrium occurs in a randomly mating population (i.e., a population in which mate selection is uninfluenced by the character in question), in which all pertinent genotypes are on the average equally viable and equally fertile. In such a population if p equals the frequency of the allele A and q equals $(1 - p)$ equals the frequency of the allele a, the frequencies of the genotypes are the terms of the expansion of $(p + q)^2$, namely, p^2 (AA), $2pq$ (Aa), and q^2 (aa). In general, if there are n alleles with frequencies p_1, $p_2, \ldots p_i \ldots p_n$, the frequencies of the several phenotypes are in accord with the expansion of $(p_1 + p_2 + \ldots p_n)$.[2] As a specific example consider the ABO alleles. The frequency of A is usually represented by p, of B by q, and O by r. The genotypes are distributed as shown in Table 4. Methods for estimating gene frequencies for some specific patterns of single gene inheritance will be demonstrated in the following paragraphs.

Two alleles with no dominance or incomplete dominance.—In this type of inheritance the heterozygote may be distinguished from either hemozygote. Some familiar examples are the MN blood type system, sickle-cell trait and disease, and Cooley's trait and disease. The three genotypes may be represented by AA, Aa and aa. Table 5A summarizes the method of estimating the gene frequencies. These estimates are maximum likelihood estimates and therefore have minimum errors of estimate in large samples. The use of these estimates to test whether the population is in a Hardy-Weinberg equilibrium is shown in Table 5B. Table 6 presents a numerical illustration of the foregoing based on unpublished data for the MN blood types of 343 unrelated individuals. The χ^2 test is used to test for goodness of fit with the expected distribution based on the Hardy-Weinberg equilibrium. (Those unfamiliar with this test will find it described in any elementary text-book on statistics.) There is only one degree of freedom, despite the three comparisons, because two restrictions have been placed on the data, namely, the fixed total and the estimate of p. Since $p + q = 1$, when p has been estimated q has also been estimated. Note that in this type of inheritance the estimate of the gene frequency is accurate regardless of the distribution of the blood types. The procedure is essentially the same in situations with three or more alleles, none of which show dominance.

Dominant alleles.—Here the three genotypes lead to only two phenotypes, the heterozygotes being indistinguishable from one of the homozygous classes—i.e., AA and Aa are phenotypically indistinguishable. The two phenotypic classes in a population in equilibrium occur with frequencies $q^2(aa)$ and $(1 - q^2)(Aa + AA)$. The frequency of allele $a = q = (q^2)^{1/2}$ and the frequency of allele $A = p = (1 - q)$. The standard error of the estimate $= [(1 - q^2)/4T]^{1/2}$ where T = the number of individuals in the sample. As an example we may take the data of Ikin *et al.* (quoted by Mourant [26], p. 366) on the frequency of the blood type P-positive in a sample of English people. P-positive individuals are believed to be PP or Pp, while P-negative individuals are assumed to be pp, hence P-negative may be considered recessive. Ikin *et al.* found

23.41 per cent of 1166 tested individuals to be *P*-negative (i.e., recessive homozygotes). The frequency of p is therefore $(.2341)^{1/2}$ or .4839. The frequency of $P = 1 - q = .5161$. The standard error of

$$p = \left(\frac{1-q^2}{4T}\right)^{1/2} = \left(\frac{1-0.2341}{4\,(1166)}\right)^{1/2} = \pm .0128\,.$$

Note, that in this example we cannot test the population against the Hardy-Weinberg distribution, because we have placed two restrictions on the sample (fixed total and fixed p [p^2]), and there are only two comparisons. The estimate of the frequency of p may, however, be used for comparison with estimates derived from other populations. Brendemoen (6) found the frequency of the allele p to be .5013 ± .0129 among 1162 Norwegians. We may ask, is the frequency of p among Norwegians and English statistically the same? The difference and its standard error are .0174 ± .0180; hence, the difference is not significant.

Sex-linked genes.—The estimate of gene

TABLE 4

EQUILIBRIUM (HARDY-WEINBERG) DISTRIBUTION OF THE *A*, *B*, *O*, GENOTYPES WITH FREQUENCIES OF ALLELES A, B, AND O EQUAL TO p, q, AND r, RESPECTIVELY $(p+q+r)^2$

Blood group	A	B	AB	O
Genotype	*AA* and *AO*	*BB* and *BO*	*AB*	*OO*
Frequency	p^2+2pr	q^2+2qr	$2pq$	r^2

TABLE 5

ESTIMATE OF GENE AND GENOTYPE FREQUENCIES, NO DOMINANCE

A. Estimate of gene frequencies

Genotype	*AA*	*Ao*	*oo*	Total
Observed number	G	H	I	T
Frequency = (no./total)	g	h	i	1

$$\text{Frequency of } A = \text{p} = \frac{2\text{g}+\text{h}}{2} = \text{g}+\tfrac{1}{2}\text{h}$$

$$a = \text{q} = \frac{\text{h}+2\text{i}}{2} = \tfrac{1}{2}\text{h}+\text{i}$$

$$\sigma = (\text{Standard error}) = \left(\frac{pq}{2\text{T}}\right)^{1/2}$$

B. Estimate of genotype frequencies

Genotype	*AA*	*Ao*	*oo*	Total
Frequency	p^2	2pq	q^2	1
Number	Tp^2	$\text{T}(2\text{pq})$	Tq^2	T

TABLE 6

EXAMPLE OF THE ESTIMATION OF THE FREQUENCY OF ALLELES WITH NO DOMINANCE

(Unpublished data for the MN groups of 343 unrelated individuals.)

GENOTYPE	*MM*		*MN*		*NN*		TOTAL	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Observed	119	34.7	155	45.2	69	20.1	343	100
Expected	112.6	32.8	167.9	48.9	62.5	18.3	343	100

$\chi^2_1 = 2.031 \quad P > .10\,.$

frequency is simplified for sex-linked recessive genes, because males have but one allele; and, hence, if penetrance is complete, the phenotype corresponds to the genotype. The frequency of a sex-linked allele may be estimated, therefore, by simply determining the proportion of males in the population who show the phenotype associated with that allele. The only two sex-linked genes that I know of for which this has been done are color-blindness and hemophilia. Unfortunately, neither of these fulfills the requirement that the phenotype be determined by a single genotype. Sex-linked color-blindness is due to at least two different genes, and so is sex-linked hemophilia. The latter is divided into classic hemophilia (AHF deficiency) and Christmas disease (or PTC deficiency). We may use the hemophilia data as an example, however, and following Graham (14) correct for the relative frequency of the two types of sex-linked hemophilia as determined by studies on relatively unselected groups of hemophiliacs.

TABLE 7

FREQUENCY OF HEMOPHILIA AND CHRISTMAS DISEASE

After Graham, 1956

	No.	Per cent
Total cases surveyed and reclassified:	247	100
Hemophilia	207	83.8
Christmas disease	40	16.2
Frequency of hemophilia in Danish males (Andereasson's survey)		4.45×10^{-4}
Adjusted frequencies for Danish males:		
Hemophilia		3.73×10^{-4}
Christmas disease		0.72×10^{-4}

Table 7 taken from Graham (14) illustrates the procedure. Other examples and applications will be found in Cotterman's review (7).

Use of consanguinity among parents to estimate gene frequency.—Various authors have derived expressions for estimating the frequency of a rare recessive gene by using the frequency of consanguineous marriages among the parents of affected individuals and among the general population. The only formulae likely to be of practical use are those for first-cousin marriages. The derivation of these and others is reviewed by Dahlberg (8, pp. 60–63).

If c_1 = the frequency with which first-cousin marriages occur,
c_2 = the frequency expected in panmixia,
q = the frequency of the recessive allele,
and k = the frequency of first-cousin marriages among the parents of affected individuals, then

$$k = \frac{c_1(1+15q)}{(c_1-c_2)(1+15q)+16q\,[1-(c_1-c_2)]}. \qquad (1)$$

If first-cousin matings occur with the expected frequency ($c_1 = c_2$),

$$k = \frac{c_1(1+15q)}{16q}. \qquad (2)$$

If the population is very large so that $c_2 \to 0$,

$$k = \frac{c_1(1+15q)}{c_1(1+15q)+16q(1-c_1)} = \frac{c_1(1+15q)}{c_1+16q-c_1q}, \qquad (3a)$$

and

$$q = \frac{c_1(1-k)}{16k-15c_1-c_1k}. \qquad (3b)$$

Note the conditions on which equations (3a) and (3b) are based, namely:

a) All occurrences of the character are genetically determined and due to the same allele;

b) the gene frequency is uniform throughout the population being sampled;

c) matings occur essentially at random;

d) all genotypes, with respect to the locus, are equally fertile on the average;

e) the expected frequency of first-cousin

marriages is essentially zero.

Assumption (*a*) is inherent in all gene frequency estimates, assumptions (*b*), (*c*), and (*d*) are assumed in all estimates of recessive genes, while (*e*) is pertinent only to the present method. This procedure requires an estimate of the frequency of first-cousin marriages in the general population and among the parents of a properly selected sample of affected individuals. It does not, however, require an estimate of the frequency of affected individuals in the population.

Although there are many sources of error in gene frequency estimates based on this equation, such estimates can provide orders of magnitude of the frequency of various genes when other preferred methods are not available. An example of the use of this method based on equation (3*b*) may be found in the paper by Neel *et al.* (30).

Average frequency of a class of mutations.—A major source of error in estimates of gene frequencies is that two or more different genes may lead to the same phenotype or to phenotypes that are difficult to distinguish. A second difficulty associated with the procedures outlined above is that only genes leading to clearly distinguished phenotypes due to simple genetic patterns may be studied. These genes compose but a very small minority of the total. Yet fundamental problems such as those having to do with genetic damage in the sense of wasted pregnancies, early post-natal deaths and generally reduced efficiency require estimates of the frequencies of large numbers of genes which, individually, do not lead to easily distinguished phenotypes. A method for estimating the average frequency of a group of such genes would indeed be highly desirable.

Muller (27), by an ingenious indirect method, estimated the average mutation rate per human gamete, the average detrimental effects of these mutations, and their average dominance. Using these estimates which were 0.1 new mutations per gamete, with an average detrimental effect of 2.5 per cent and an average dominance of 5 per cent, Muller estimated that each individual, on the average, carries eight slightly dominant, detrimental mutant genes in heterozygous condition. Detrimental effect here means the average reduction in fertility (the number of offspring surviving to reproduce) caused by the gene in the homozygous condition; average dominance refers to the average reduction in fertility caused by the gene in the heterozygous condition divided by that caused by the gene in the homozygous condition (i.e., detrimental effect when heterozygous/detrimental effect when homozygous.)

As Muller pointed out, more direct methods of determining the average frequency of detrimental genes in the population are desirable.

S. C. Reed (35) indicated that the offspring of father-daughter or brother-sister matings could be used to estimate the frequency in the population of heterozygosis for a group of known recessive genes. The procedure rests on the genetic fact that the probability that a daughter will carry a recessive gene for which her father or brother is heterozygous is one-half. Hence, the probability that a father-daughter mating would yield a child homozygous for a recessive gene present in the father is one in eight (i.e., the probability that she is heterozygous [$\frac{1}{2}$] times the probability of a homozygous recessive offspring from the mating of two heterozygotes). It is possible with this procedure to compute the average number of genes for which the father is heterozygous as a function of the frequency with which such offspring are homozygous for recessive genes.

Slatis (38) extended the method to first-cousin marriages which are probably more frequent than incestuous matings. He provided a method for taking family size into account and for combining data from families of different sizes. His method is as follows:

Let s = size of family;

$z_s = 1 - (\frac{3}{4})^s$ = the probability of at least one affected child in a

family of size s with heterozygous parents,

r_s = the number of recessive genes in families of size s,

n_s = the number of families size s.

and c = the average number of recessive genes for which the parents of these families are heterozygous.

Then $z_s c$ mutants will be seen per family of size s, and $n_s z_s c = r_s$, mutants will be seen in n_s families of size s, hence

$$c = \frac{r_s}{n_s z_s}.$$

In the total sample, the families of various sizes will have an average of

$$\bar{c} = \frac{\Sigma_s r_s}{\Sigma_s n_s z_s} \text{ mutants per family .}$$

The average number of genes for which *each pair* of common grandparents is heterozygous equals $16(c)$, because the probability that two first cousins will each inherit the same gene from among the four alleles in the common grandparents is 1 in 16. Hence, *each* of the common grandparents is heterozygous for $8(c)$ genes. Penrose (33) applied a similar method of analysis to data collected by Böök (5) in a North Swedish population. The correct estimate from these data is that each individual is heterozygous for about six mutants. This estimate is lower than Muller's, but it considers only major deviations known or suspected to be due to recessive genes.

Morton, Crow, and Muller (25), have extended the method to provide an estimate of the number of "lethal equivalents" present in the heterozygous condition in the average person. The lethal equivalent value of an allele equals the value of the selection coefficient (s) against the homozygote for that allele. Thus, an allele which causes death in all homozygotes (s equals one) is said to equal one lethal equivalent. An allele which causes death in half the homozygotes ($s = .5$) is said to equal .5 of a lethal equivalent. The actions of the several genes are assumed to be independent (non-synergistic [27]), but their effects are assumed to be additive. Hence, a gamete with two mutants, each of which would cause death in 50 per cent of those zygotes homozygous for it, may be said to contain one lethal equivalent.

In essence, the method developed by Morton *et al.* (25) consists of dividing the causes of death into two portions: One, called 'A', represents deaths due to environmental conditions plus those due to homozygosity and heterozygosity resulting from non-consanguineous matings; while the other, called 'B', equals deaths due to homozygosity resulting from inbreeding minus the genetic deaths represented by 'A.' 'A' and 'B' are evaluated as follows:

Let

F = the coefficient of inbreeding (48). It is the probability that both alleles at a locus have been derived from one present in a common ancestor; that is, they are identical by descent (Li and Sacks, 1954);

s = the selection coefficient against the homozygote; that is, the proportionate reduction in the relative fertility of homozygotes;

h = a measure of dominance; it is the fraction of the selection coefficient(s) expressed against heterozygotes;

S = the probability of surviving.

Sewall Wright (48) has shown that, in a population with an inbreeding coefficient F, the three genotypes for a given locus occur with the following frequencies (see Li [21], chapter 11 for review):

Genotype	$A\,A$	$A\,a$	$a\,a$
Frequency	$F(1-q) + (1-F)(1-q)^2$	$2(1-F)\,q\,(1-q)$	$Fq + (1-F)\,q^2$

Where $F(1-q)$ and Fq equal the frequencies of homozygosis because of inbreeding, for the dominant and recessive alleles, respectively, and the remaining terms represent the frequencies of the indicated genotypes which occur in the absence of inbreeding.

Therefore, the probability that an allele with frequency q will cause death in a population with a coefficient of inbreeding equal to F, selection pressure against homozygotes for the allele equal to s, and against heterozygotes for this allele equal to hs is

$$\underset{\text{homozygotes}}{qFs+q^2(1-F)\,s}+\underset{\text{heterozygotes}}{2q(1-q)(1-F)\,sh}\ ,$$

where the relative survival value of the homozygous dominant is one.

The probability of surviving genetic causes of death is one minus the above quantity, or

$$1-qFs-q^2s+q^2Fs-2q(1-q)\,sh + 2q(1-q)Fsh\ .$$

Death may occur for non-genetic (environmental) reasons. Let the probability of death due to a particular environmental cause be x, and be independent of the genotype. The probability of not succumbing to environmental causes (surviving) is therefore $(1-x)$. The probability of surviving despite a given environmental risk and the genetic risk due to a specific allele is therefore:

$$(1-x)\,[1-qFs-q^2s+q^2Fs - 2q(1-q)\,sh+2q(1-q)Fsh]\ ,$$

which on expansion equals

$$1-qFs-q^2s+q^2Fs-2q(1-q)\,sh + 2q(1-q)Fsh-x\ ,$$

plus a series of terms involving the product of x and the terms other than one in the bracket. Since x and each of these terms are small, their products may be ignored without introducing great error into what is at best an approximate calculation.

The probability of dying is one minus the above quantity or

$$x+q^2s+2q(1-q)\,sh+qFs-q^2Fs - 2q(1-q)Fsh\ .$$

The over-all probability of death from any environmental cause or any locus is the sum of these terms and

$$=\Sigma x+\Sigma q^2s+2\Sigma q(1-q)\,sh + F[\Sigma qs-\Sigma q^2s-2\Sigma q(1-q)\,sh]\ .$$

Morton *et al.* (23), let:

$$A=\Sigma x+\Sigma q^2s+2\Sigma q(1-q)\,sh\ , \quad \text{and}$$

$$B=\Sigma qs-\Sigma q^2s-2\Sigma q(1-q)\,sh\ .$$

Hence, the probability of death from some cause may be written as

$$A+BF$$

The first term of the Poisson distribution expresses the probability that an event with a given mean expectancy (in this case $A+BF$) will not occur. The event we are discussing is death. If death does not occur, survival does, hence the probability of survival (S) may be stated as the first term of a Poisson distribution and

$$S=e^{-(A+BF)} \quad \text{or} \quad -\log_e S=A+BF$$

The value of F is computed for each degree of consanguinity observed in the sample. The survival rate is observed for the offspring resulting from marriages of each degree of consanguinity. These values are used to estimate the values of A and B. Note that B is less than Σqs, the mutational damage per gamete, and that $A+B=\Sigma qs+\Sigma x$ is greater than the Σqs. Hence, the number of lethal equivalents per gamete is somewhat less than $A+B$ and more than B.

Using data from three different sources, which among them included data on stillbirths and post-natal deaths up to age 20,

Morton, Crow, and Muller estimated that the average individual is heterozygous for three to five lethal equivalents. The authors believe their estimate is probably too low, because the data omit abortions, early adult deaths, and cases of infecundity. Since a lethal equivalent may comprise two or more detrimental mutants, each individual is, on the average, probably heterozygous for more than five genes. Morton *et al.* (23) computed also that the average person carries an additional four to five genes which, if homozygous, could cause conspicuous abnormality and probably reduced reproductive potential.

MUTATION RATES

The mutation rate of a specific dominant gene may be estimated by determining the incidence of affected individuals whose parents were not affected. This method, which is known as the direct method, assumes complete penetrance of the dominant gene. If penetrance is incomplete, a correction based on an estimate of the probability that a phenotypically normal individual heterozygous for the gene will have had only one affected child among '*s*' offspring must be introduced. In either situation it is assumed that all cases are due to the same locus and, incidentally, that all are offspring of their legal parents.

Alternately, the mutation rate may be estimated by the indirect method which may also be used to estimate the mutation rate of recessive alleles. All indirect methods of estimating mutation rates depend upon the assumption that the population is in equilibrium, i.e., that the frequency of the allele in question remains constant from generation to generation. If this assumption is correct, the frequency of origin of the allele by mutation must equal the frequency of loss of the allele by reduced fertility of a portion of those carrying it (see Muller [27] for a detailed discussion). Additional assumptions are: (*a*) all instances of the character in question are genetically determined and due to the same locus; and (*b*) for recessive alleles, mating is at random with respect to the locus in question.

Table 8, modeled after the one published by Penrose (32), presents a summary of the indirect methods for estimating the mutation rates of autosomal dominant and recessive genes and sex-linked recessive genes (see Stern [41], pp. 408–12, for lucid and simple derivations of these methods). It should be noted that all methods ignore reverse mutations, i.e., if the allele under consideration is dominant (*A*), mutation from '*a*' to '*A*' is examined, but mutation from '*A*' to '*a*' is not.

TABLE 8

SUMMARY OF INDIRECT METHODS FOR ESTIMATING THE MUTATION RATES OF INDIVIDUAL GENES

Steps in calculation	Rare dominant trait	Sex-linked trait	Recessive trait
1. Sex affected	M or F	M	M or F
2. Genotype of affected	Aa	a	aa
3. Frequency of genotype in population	$2p$	q	q^2
4. Relative loss of fitness	$(1-f)$	$(1-f)$	$(1-f)$
5. Mutation rate/gamete to allele causing genotype	$(1-f)p$	$\frac{1}{3}(1-f)q$	$(1-f)q^2$

Step three (Table 8) indicates that it is necessary to determine the frequency of affected individuals in the general population. This involves an enumeration of all affected individuals in a given region and an enumeration of the total population of which they form a part. There is room for error in both these enumerations. The numerator (the number of affected) may be underestimated because some diagnosed cases were missed, or because some cases have not been diagnosed, or have been misdiagnosed, and so on. It may be overestimated if some cases are not genetically determined or are due to different genes. Errors in

the denominator (size of the total population) may result from any one of several reasons, among the most important are the use of an incorrect base population (in time or location, or both), and faulty census data. Neel (29) has reviewed and illustrated these difficulties with respect to three dominantly inherited, rare diseases.

The frequency of affected individuals is used to estimate the frequency of the allele in the population. In a population mating at random, the frequency of the heterozygote is $2pq$. Since $q = (1 - p)$ we may write this as $2p(1 - p)$ or $2(p - p^2)$. In the case of a rare dominant p is small (less than .001), hence p^2 is very small. Therefore $2p(1 - p)$ is very nearly equal to $2p$. Step 4 indicates that the relative fertility (f) of affected individuals must be determined. In practice this is based on the average number of surviving offspring per affected individual divided by the average number of offspring per unaffected individual of a comparable socio-economic, ethnologic, etc., group. Some aspects of the problems of estimation involved here have been reviewed by Krooth (19), and Reed and Neel (36).

If f is the relative reproductive fitness of affected individuals, $(1 - f)$ is the relative loss in reproductive fitness of such individuals. For every individual lost one dominant gene is lost. This lost gene must be replaced by one of the two genes which give rise to a new individual; hence, the rate of replacement of genes required for equilibrium equals $(1 - f)p$ (recall that $2p$ equals the frequency of dominant individuals).

We shall consider the estimate of the mutation rate of the gene causing retinoblastoma as an example of the method of estimating the mutation rate of a dominant gene and of the pitfalls awaiting even highly skilled workers well aware of the sources of error. In a very careful study of retinoblastoma in Michigan, Falls and Neel (11), using the direct method, estimated the mutation rate of the normal allele causing retinoblastoma as 2.3×10^{-5}. This estimate, as the authors pointed out, was based on the assumption that all retinoblastoma is genetically determined and due to the same gene with essentially complete penetrance. A mutation rate of 1.6×10^{-5} may be derived by using the authors' data for the incidence of the disease and their estimate of the relative reproductive fertility of retinoblastoma parents. This is not very different from the rate derived by Philip and Sorsby (34) from data collected in London, namely, 1.4×10^{-5}. As we shall see, all these estimates are probably too high. Vogel (43), from a consideration of the proportion of cases which are hereditary (i.e., have affected ancestors) and the relative fertility of retinoblastoma patients, concludes that only 25 per cent of all sporadic cases represent mutations, the remaining 75 per cent being phenocopies (environmentally induced non-hereditary copies of phenotypes which are known to be genetically determined) or somatic mutations.

Tucker, Steinberg, and Cogan (42) and Vogel (44) independently checked this point by determining the frequency of retinoblastoma among the offspring of individuals who had survived the disease and who had no known collateral or ancestral relatives with retinoblastoma. These direct studies indicate that about 20 per cent of the sporadic cases are due to new mutations; the remainder appear to be non-genetic. Vogel (44) estimates the mutation rate to be 6 to 7×10^{-6}, i.e., about one quarter the original estimate of Falls and Neel (11) and about half the rate estimated by Philip and Sorsby (32).

These several papers serve to illustrate, forcibly, the difficulties confronting those who would study mutation rates and the necessity for caution in comparing estimates of the mutation rates of specific genes computed for different populations.

We have already discussed the difficulty encountered in studying the frequency of the gene causing hemophilia, namely, that clinical hemophilia has been shown in the laboratory to be a group of diseases. Genetic evidence shows that two of these, classical hemophilia (AHF deficiency) and Christmas disease (plasma thromboplastin component, PTC, deficiency), are due to sex-

linked genes (review in Graham [14]). Clearly, the earlier calculations of the mutation rate for hemophilia will have to be reevaluated. It is not all certain, however, that all cases of AHF deficiency are due to the same allele; hence, even the separation of sex-linked hemophilia, into the two categories of AHF deficiency and Christmas disease, will not insure an accurate estimate of the mutation rate.

The estimate of the mutation rate for an autosomal recessive mutant has, in addition to the complications already enumerated and illustrated, the difficulty that the gene may not be completely recessive and that the survival value of the heterozygote may not be identical with that of the homozygous normal. This can introduce considerable error into the estimate, because most of the recessive alleles in the population will be carried by heterozygotes, when the recessive allele is less frequent than the dominant allele. For practical purposes this is true of all diseases for which mutation rates would be calculated. As an illustration, consider a relatively frequent recessive disease, one with a frequency of .01. About nine times as many recessive alleles would be carried by heterozygotes as by homozygotes. Hence, any deviation from complete recessivity may be expected to have a marked influence on the mutation rate required to maintain the population at equilibrium for such an allele. If the heterozygote is more viable than either homozygote, a situation postulated for the sickle-cell trait (see Allison [2] for review), a lower rate of mutation is required to maintain equilibrium; if the heterozygote is less fit than the homozygous dominant, a higher mutation rate is required to maintain equilibrium. These problems have been recognized and discussed by many authors. A relatively recent discussion of them may be found in a paper by Neel (28).

An alternative to estimating the mutation rate of specific genes is to estimate the average mutation rate of a group of genes causing a reduction of fitness of those carrying them. Morton, Crow, and Mulier (25) showed that the method they developed to estimate the average number of such genes carried by an individual offers a means of doing this. It is assumed, as in all indirect estimates of mutation rates, that the population is at equilibrium with respect to the frequency of these alleles. Morton *et al.* proceeded as follows:

Let Fs = the probability of a given mutant's being eliminated through homozygosity resulting from inbreeding;

$(1 - F)qs$ = the probability of a given mutant's being eliminated through homozygosity resulting from its meeting a previously existing allele;

$(1 - F)(1 - q)sh$ = the probability of a given mutant's being eliminated in the heterozygous condition.

The total probability of elimination is the sum of these probabilities or approximately $Fs + qs + sh$ (terms such as Fqs, Fsh, and $Fqsh$ being omitted, because they are small compared with those retained). The above expression may be written as $(F + q + h)s$, and by designating $F + q + h$ as z; as zs. If zs is the probability that a mutant allele will be eliminated in any given generation, the average number of generations it will exist in the population before it is eliminated (the mean persistence) equals $1/zs$. The frequency of the allele in the population (i.e., the probability of its occurring in a gamete) will equal μ/zs, where μ is the mutation rate. Recall that s equals the fraction of a lethal equivalent represented by a given allele. Then an allele with frequency μ/zs and a lethal equivalent value of s will contribute $(\mu/zs)s$, or μ/z lethal equivalents per gamete. The number of lethal equivalents contributed by all alleles may be found by summing over all loci; i.e., the total number of lethal equivalents per gamete equals $\Sigma(\mu/z)$. Under certain conditions $\Sigma(\mu/z)$ may be written as $(\Sigma\mu)(\overline{1/z})$ (23). Hence, the total mutation rate per gamete ($\Sigma\mu$) may be estimated from the estimate of the number of lethal equiv-

alents per gamete if a value for $\overline{1/z}$ (the harmonic mean of z) can be obtained. Using data from Drosophila, Morton *et al.* (25) consider .02 to be a reasonable estimate of the harmonic mean of z. Using this value and the previously derived value of 1.5 to 2.5 lethal equivalents per gamete, they arrived at total mutation rate of alleles causing death between late fetal and early adult life of .03 to .05 per gamete per generation. The total detrimental mutation rate, including that of genes causing early embryonic death, is estimated to be 2–3 times as great, i.e., from .06 to .15 per gamete.

This method promises to be most useful in determining gene frequencies and mutation rates. It seems to be the best method for obtaining the data required to evaluate the effect of mutagens on man. Better and more extensive data based on human material are required for each of the applications. We are all obligated to help supply such data whenever we can.

Only some of the more important or more commonly used methods for the analysis of hereditary patterns, the determination of gene frequencies and of mutation rates have been discussed. There has not been time to discuss such important problems as the measurement of linkage, the determination and measurement of factors influencing the frequencies of genes in populations, and so on. The interested reader will find references to literature on these topics in *The American Journal of Human Genetics*.

It has been my purpose to expose you to some of the patterns of thought and methods of approach used by geneticists in solving problems of heredity in man. If this paper has opened the door to these problems for you, I am pleased; if in addition it has given you some insight into how these problems are handled, I have accomplished my purpose, and I am delighted.

REFERENCES

1. ALDRICH, R. A.; STEINBERG, A. G.; and CAMPBELL, D. C. Pedigree Demonstrating a Sex-linked Recessive Condition Characterized by Draining Ears, Eczematoid Dermatitis and Bloody Diarrhea. Pediatrics, **13**:133–39, 1954.
2. ALLISON, A. C. Aspects of Polymorphism in Man. Cold Spring Harbor Symp. Quant. Biol., **20**:239–55, 1955.
3. BAILEY, N. T. J. The Estimation of the Frequencies of Recessives with Incomplete Multiple Selection. Ann. Eugen., **16**:215–22, 1951.
4. ———. Classification of Methods of Ascertainment and Analysis in Estimating the Frequencies of Recessives in Man. *Ibid.*, pp. 223–25.
5. BÖÖK, J. A. Genetical Investigations in a North Swedish Population. The Offspring of First-Cousin Marriages. Ann. Human Genet., **21**:191–221, 1957.
6. BRENDEMOEN, O. J. Blood Factor P in Eastern Norway. Acta path. microbiol. Scandinav., **27**:338–39, 1950.
7. COTTERMAN, C. W. Estimation of Gene Frequencies in Nonexperimental Populations. *In:* KEMPTHORNE, BANCROFT, GOWEN, and LUSH (eds.), Statistics and Mathematics in Biology. Ames, Iowa: The Iowa State College Press, 1954.
8. DAHLBERG, G. Mathematical Methods for Population Genetics. New York: Interscience Publishers, 1948.
9. DAVID, P. R. *In:* Conference on Problems and Methods in Human Genetics. Am. J. Human Genet., **6**:51–194, 1954.
10. DAVID, P. R., and SNYDER, L. S. Genetics and Disease. *In:* Proc. Second Nat. Cancer Conf., pp. 1128–38. New York, Am. Cancer Soc., 1954.
11. FALLS, H. F., and NEEL, J. V. Genetics of Retinoblastoma. Arch. Ophthalmol., **46**: 367–89, 1951.
12. FINNEY, D. J. The Truncated Binomial Distribution. Ann. Eugen., **14**:319–28, 1949.
13. FISHER, R. A. The Effect of Methods of Ascertainment upon the Estimation of Frequencies. Ann. Eugen., **6**:13–25, 1934.
14. GRAHAM, J. B. Biochemical Genetics of Blood Coagulation. Am. J. Human Genet., **8**:63–79, 1956.
15. HALDANE, J. B. S. The Estimation of the Frequencies of Recessive Conditions in Man. Ann. Eugen., **8**:255–62, 1938.
16. HOGBEN, L. An Introduction to Mathematical Genetics. New York: W. W. Norton & Co., Inc., 1946.

17. HUNTLEY, C. C. and DEES, S. C. Eczema Associated with Thrombocytopenic Purpura and Purulent Otitis Media. Pediatrics, **19**:351–61, 1957.
18. IKIN, E. W.; KOPEC, A. C.; MOURANT, A. E.; PARKIN, D. M.; and WALBY, J. A. B., 1952. Unpublished data quoted in Mourant, 1954.
19. KROOTH, R. The Use of the Fertilities of Affected Individuals and Their Unaffected Sibs in the Estimation of Fitness. Am. J. Human Genet., **7**:325–60, 1955.
20. LI, C. C. Segregation of Recessive Offspring. Methods Med. Research, **6**:3–16, 1954.
21. ———. Population Genetics. Chicago, Ill.: University of Chicago Press, 1955.
22. LI, C. C., and SACKS, L. The Derivation of Joint Distribution and Correlation between Relatives by the Use of Stochastic Matrices. Biometrics, **10**:347–60, 1954.
23. MORTON, N. E. Segregation Analysis in Human Genetics. Science, **127**:79–80, 1957.
24. ———. Genetic Tests under Incomplete Ascertainment. Am. J. Human Genet., **11**: 1–16, 1959.
25. MORTON, N. E.; CROW, J. F.; and MULLER, H. J. An Estimate of the Mutational Damage in Man from Data on Consanguineous Marriages. Proc. Nat. Acad. Sc., **42**:855–63, 1956.
26. MOURANT, A. E. The Distribution of the Human Blood Groups. Springfield, Ill.: Charles C Thomas, 1954.
27. MULLER, H. J. Our Load of Mutations. Am. J. Human Genet., **2**:111–76, 1950.
28. NEEL, J. V. The Study of Human Mutation Rates. Am. Nat., **86**:129–44, 1952.
29. ———. Problems in the Estimation of the Frequency of Uncommon Inherited Traits. Am. J. Human Genet., **6**:51–60, 1954.
30. NEEL, J. V.; KODANI, M.; BREWER, R.; and ANDERSON, R. C. The Incidence of Consanguineous Matings in Japan. With Remarks on the Estimation of Comparative Gene Frequencies and the Expected Rate of Appearance of Induced Recessive Mutations. Am. J. Human Genet., **1**:156–78, 1949.
31. NEEL, J. V., and SCHULL, W. J. Human Heredity. Chicago, Ill.: The University of Chicago Press, 1954.
32. PENROSE, L. S. *In:* The Hazards to Man of Nuclear and Allied Radiations. London: Her Majesty's Stationary Office, 1956.
33. ———. A Note on the Prevalence of Genes for Deleterious Recessive Traits in Man. Ann. Human Genet., **21**:222–24, 1957.
34. PHILIP, U., and SORSBY, A. Quoted in Falls and Neel, 1951.
35. REED, S. C. A Test for Heterozygous Deleterious Recessives. J. Hered., **45**:17–18, 1954.
36. REED, T. E., and NEEL, J. V. A Genetic Study of Multiple Polyposis of the Colon (with an Appendix Deriving a Method of Estimating Relative Fitness). Am. J. Human Genet., **7**:236–63, 1955.
37. REESE, A. B. Frequency of Retinoblastoma in the Progeny of Parents Who Have Survived the Disease. Arch. Ophth., **52**:815–18, 1954.
38. SLATIS, H. A Method of Estimating the Frequency of Abnormal Recessive Genes in Man. Am. J. Human Genet., **6**:412–17, 1954.
39. SNYDER, L. S., and DAVID, P. Penetrance and Expression. *In:* Clinical Genetics. St. Louis, Mo.: C. V. Mosby Co., 1953.
40. STERN, C. The Hardy-Weinberg Law. Science, **97**:137–38, 1943.
41. ———. Principles of Human Genetics. San Francisco, Cal.: W. H. Freeman & Co., 1949.
42. TUCKER, D. P.; STEINBERG, A. G.; and COGAN, D. G. Frequency of Genetic Transmission of Sporadic Retinoblastoma. Arch. Ophth., **57**:532–35, 1957.
43. VOGEL, F. Über genetik und mutationsrate des retinoblastoms. Ztschr. menschl. Vererb., **34**:308–36, 1954.
44. ———. Neue untersuchungen zur genetik des rentinoblastoms (glioma retinae). *Ibid.*, **34**:205–36, 1957.
45. WEINBERG, W. Mathematische grundlagen der probandenmethode Ztschr. Indukt. Abstamm. Vererb., **48**:179–228, 1927.
46. WINTERS, R. W.; GRAHAM, J. B.; WILLIAMS, T. F.; MCFALLS, V. W.; and BURNETT, C. H. A Genetic Study of Familial Hypophosphatemia and Vitamin D Resistant Rickets. Trans. Assoc. Am. Physicians, **70**: 234–42, 1957.
47. WOLFF, J. A., and BERTUCCIO, M. A Sex-linked Genetic Syndrome in a Negro Family Manifested by Thrombocytopenia, Eczema, Bloody Diarrhea, Recurrent Infection, Anemia, and Epistaxis. J. Dis. Child., **93**:74, 1957.
48. WRIGHT, S. Coefficients of Inbreeding and Relationship. Am. Nat., **56**:330–38, 1922.

Importance of Genetics of Viruses in Medical Research

HILARY KOPROWSKI, M.D.
The Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

There was a young lady named Rood,
Who was such an absolute prude,
That she pulled down the blind
When changing her mind,
Lest a curious eye should intrude.
—*The World's Best Limericks* (Mount Vernon, N.Y.: Peter Pauper Press)

Milnes était un garçon d'esprit qui faisait, et ce qui est plus rare, qui disait beaucoup de bêtises.
—ALEXIS DE TOCQUEVILLE, *Souvenirs*

When Doctor Lederberg flattered my ego by assigning to me the noble task of discussing the importance of virus genetics in medical research, the situation smacked of the one described by Sir Osbert Sitwell in his *Noble Essences*. In this anecdote, a robust old country neighbor was heard during a severe thunderstorm to address his faithful and aging servant: "Alec, you damn fool, don't stand about there doing nothing. Climb up the lightning conductor, can't you, and see if it's working." Thus "Climbing up the Lighting Conductor" becomes the first motto of this presentation.

The second has been provided by one of our "missile" generals testifying before a congressional committee during the post-Sputnik and pre-Explorer era. This general, when asked how our modern weapons compared with those of the Russians, replied: "We must catch up with them, but of course we are ahead of them." This is quite apt to the position of mammalian viruses *vis-à-vis* the genetics of phages. We are being exhorted to catch up with the phage geneticists; we have been subjected to merciless drubbing for the past decade because of the inexact methods we employ, because we show poor scientific judgment, and so on and so forth (and how true), and yet we are ahead of them because, while the phage geneticists were still playing around with phage-shockates that perhaps are pure DNA (though it is still doubtful) but which invade the bacteria only when the wall is removed, we are ahead of them because we discovered a mammalian virus RNA which penetrates intact cells.

The third motto is that of Marcus Annaeus Lucanus: "Metiri sua regna decet, vires-que fateri." Since all of you are familiar with Latin, you no doubt are sure that "vires" refers to viruses. Nothing could be further removed from the truth—and yet curiously enough this motto, too, is quite pertinent to our subject, for it means:

It Is Fitting To Measure His Own Powers, and To Admit His Strength.

. . . What such haste
Is yours, as that it cannot wait
fit times. . . .
—JOHN FORD, " 'Tis a Pity She's a Whore," in *Five Plays* (New York: Hill & Wang, Inc., 1957)

REMEMBRANCE OF THINGS PAST

Froissart's observation that "the most profitable thing in the world for the understanding of human life is history" can also be applied to the events which have led to the present-day concept of virus stability and virus mutation.

In what now seems like the historical past, the "subconscious" mind was appar-

ently groping toward virus genetics but had concentrated its attention on the one of the then easily discernible characters of a virus —its pathogenicity for the human species. All the early attempts at controlled manipulation of a virus had one objective in mind: to modify its disease-producing characteristics but at the same time to retain its immunizing power. This *leitmotiv* is encountered, with numerous variations, throughout the successive studies of viruses. Even today many of the detailed analyses of genetic properties were impelled by the desire to find a virus with definable characters which would serve to protect man or animal against disease.

Paradoxically, a variant of an otherwise highly virulent virus with the longest history has left for posterity very little to lean on. There is no doubt that Jenner used cowpox for human vaccination in his original investigations, but subsequent maintenance of the virus by man-to-man passages led to its contamination with smallpox (12, 35). Thus, although we can determine the character of the vaccinia virus employed for prophylactic measures, we are unable to define the origin of the virus. Repeated passages of cowpox in laboratory animals lead to a strain with vaccinia properties (3), but the occurrence of natural variants (13) would suggest that vaccinia may have arisen as a stable mutant in strains of cowpox.

The first cognitive attempt at manipulation of a virus, to obtain variation of its character, was done by Pasteur. Through passage in rabbits, the character of the wild type, or the so-called street virus of rabies, had undergone considerable change. Further variation in qualities was observed after passage in the developing chick embryo (32). The characteristics of the street virus and its three descendants (Table 1) range from complete absence of virulence of the HEP Flury strain, for any adult mammal, to full virulence of the street virus for any warm-blooded animal. The latter character is associated with the capacity of the virus to multiply *in vivo* in the salivary gland tissue. It should be mentioned at this point that rabies virus, because of its characteristics, is singularly unfitted for genetic study in which pure lines originating from a single virus particle must be employed.

TABLE 1

CHARACTERS OF FOUR VARIANTS OF RABIES VIRUS

RABIES STRAIN	INCUBATION PERIOD	PRESENCE SALIVARY GLAND	VIRULENCE Rabbit i.c.	Rabbit e.n.	Mouse i.c.	Mouse e.n.	Dog i.c.	Dog e.n.	Cow i.c.	Cow e.n.
Street	Long, variable	+++	+++	+++	+++	+++	+++	+++	+++	+++
Fixed	Short	0	+++	+	+++	++	+++	+	+++	++
Flury LEP	Short	0	++	0	+++	±	±	0	+	+
Flury HEP	Short	0	0	0	0*	0	0	0	0	0

i.c. = Intracerebral inoculation.
e.n. = Extraneural inoculation.
0 to +++ = Nil to very high.

* Virulent for newborn mice only.
NOTE: All fixed strains, and many of the street strains, grow in chick embryo.

Another historical milestone was the emergence of the 17D strain of yellow fever virus (46) after passage of a virulent strain in mouse brain and in chick embryo tissue cultures. Continuous cultivation of the 17D strain, however, brought about one other, this time highly undesirable, character—loss of the immunizing property. While examining the data presented in Table 2, which by no means should be construed as being an exhaustive study of all the properties of the yellow fever strain, one is amazed at the paucity of defined markers for a virus like the 17D strain, which has been used, and is being used, for immunization of millions of people against yellow fever.

There are other "historic" examples of more or less stable variants of viruses produced by laboratory manipulation. This type of research is motivated to a great ex-

tent by the desire to decrease virulence of a virus for an economically important host, and is characterized chiefly by its unpredictability. No attempts were made, and no attempts could have been made because of lack of suitable techniques, to work with anything other than heterogenous viral populations. This led to selection of properties usually unreproducible for the second time. A notable exception was the study of the vaccinia virus (13).

The variations in influenza viruses are by now also history. However, in this case the less my arguments would fail without bringing forth the "flu and its variants."

There is an active tedium just as
there is a passive one.
—HELMUT KUHN, *Encounter with Nothingness*
(London: Methuen & Co., 1951)

MARKERS—MARKERS—MARKERS

Viruses as antigenic entities evoke serologic reactions which are characteristic of their immunological identity. Performance of the serologic tests is quite cumbersome;

TABLE 2

SOME DISTINGUISHABLE MARKERS OF THE ORIGINAL AND TWO DESCENDANT STRAINS OF YELLOW FEVER VIRUS

	Virulence			
	Man	Monkey		Immunization
Strain		i.c.	e.n.	of man
Asibi	++	+++	+++	+++
17D	0	±	0	+++
17DD High	0	?	0	± or 0

i.c. = Intracerebral inoculation.
e.n. = Extraneural inoculation.
0 to +++ = Nil to very high.

change from the human to the laboratory form (chick embryo cultivated) has been minutely studied and stands out as the first purposeful attempt at genetic analysis of inheritable characteristics in a mammalian virus (4, 6, 7). In brief, the influenza virus isolated from man (the O form, Table 3) can be cultivated after inoculation into the amniotic sac of fertile hens' eggs. In addition, this virus will display other properties, summarized in Table 3. In contrast, the virus which has been manipulated sufficiently to grow in the allantoic cavity (D form) displays entirely different characteristics (Table 3). If a fresh human isolate is maintained in cultures of human embryonic lung, it retains the property of the O form (37).

There are numerous reports on studies of influenza and other myxoviruses with descriptions of genetic markers and genetic analyses of the inheritance of demonstrable characteristics. Several reviews have been published recently; therefore, I shall abstain from dipping into the "influenza book," un-

TABLE 3

MARKERS OF THE TWO FORMS OF INFLUENZA*

	O Form	D Form
RBC Agglutination:		
Human cells	+++	+++
Guinea pig cells	+++	+++
Chicken cells	0	+++
Site of Infection:		
In chick embryo	Amnion	Allantois
Reaction with ovo-mucins	0	+++
Pathogenicity for man	++	0

* After Burnet (4).

and were this the only method available for the genetic study of viruses, progress in this field would be very slow indeed. It is not the purpose of this review to assemble all characteristic markers of many viruses, and only a few examples will be discussed which, because of their relative simplicity, could be used for investigation of genetic constancy of a given virus strain.

The ether and bile salt resistance permits classification of viruses into four unequal groups (Table 4) (2, 43, 47, 49): (*a*) Viruses inactivated by ether and desoxycholate form

the largest group—it comprises the myxoviruses (including mumps, influenza, Newcastle, etc.); Rous sarcoma and most of the encephalitides which belong to the serologically distinct group called Arbor A and B (8); (*b*) the pox viruses and human and mouse polio belong to the ether and desoxycholate resistant group; (*c*) psittacosis is inactivated by ether but not by DCA; and (*d*) rabies behaves in an opposite way.

of different markers among the 23 strains investigated (Table 6). Fenner divided his strains into two large groups: those producing small nodular lesions without necrosis, called the dermal strain, and those causing a large, flat lesion with extensive necrosis. Of the 23 studied only two strains of cowpox producing white pocks shared all other markers. Four strains differed only in relation to one character: pathogenicity for

TABLE 4

SUSCEPTIBILITY OF VIRUSES TO ETHER AND BILE-SALTS

DCA+ ET+	DCA− ET+	DCA− ET−	DCA+ ET−
Myxoviruses	Psittacosis	Pox viruses	Rabies
		Poliovirus	
Rous sarcoma		Mengo*	
		Mouse enceph.	
Rift Valley fever			
Eastern eq. enc.			
Western eq. enc.			
Louping ill			
Yellow fever			
St. Louis enc.			

DCA+ = inactivated by sodium desoxycholate.
DCA− = sodium desoxycholate resistant.
ET+ = inactivated by ethyl ether.
ET− = resistant to ethyl ether.

* = ether sensitivity not known.
See References 2, 43, 47, 49.

TABLE 5

CRITERIA OF CHARACTERS OF THREE POX VIRUSES

Pock appearance
Pock size
Viral content of pock
Virulence for mice
Infectivity for rabbits
Hemagglutinins
Heat resistance

A more sophisticated report on genetic markers has been presented by Fenner (20) for three pox viruses: vaccinia, cowpox, and rabbit pox. Eight different characters related to pock appearance, size, and infectivity, virulence for mice and rabbits, hemagglutinin production, and temperature susceptibility were studied (Table 5). The variability in the biological characters of the virus is best illustrated by the distribution

mice. In general, intracerebral virulence for mice and rabbits was associated with a red pock, and large hemorrhagic lesions were seen after intradermal inoculation of rabbits. Most of the strains, but not all, produced hemagglutinin, and most were heat-resistant.

To a great extent research on genetic markers of the poliomyelitis virus had the same motivation, that of decreasing its virulence, but the results of such studies have contributed greatly to the accumulation of basic data in the field of virus genetics.

Table 7 presents six different markers of polioviruses. The first refers to observations made by Dulbecco and Vogt (18) on the larger-sized plaques which appeared in the course of rapid passages of Brunhilde Type I virus on monolayer of renal epithelium from cynomolgus monkeys. This technique is em-

ployed widely in the study of quantitative aspects of virus growth, and the principal steps involved will be described briefly. A cell suspension is obtained either from organs (e.g., lung, kidney, etc., of adult animals) or from whole chick embryo or mouse embryo, and the cells are dispersed with the help of enzymatic digestion by trypsin or versene. A count of viable cells is made, and then the cells, resuspended in nutritive medium, are placed in petri dishes and exposed to virus infection. At given time intervals the virus is removed and the cellular monolayer is covered with an agar overlay, which is allowed to solidify; it is then incubated at 37° C. in the presence of CO_2, which helps to maintain the pH of the cultures at the desired levels.

The virus particles which had been adsorbed penetrate into the cells, multiply, and cause cellular necroses. These necrotic foci, called plaques, remain unstained areas among cells which take up neutral red stain as a sign of their viability. With this system, the multiplicity of infection (that is, the relationship between cells exposed to a given number of virus particles), the latent period (i.e., the time interval between adsorption and the release of first virus particle), and yield of virus (i.e., the number of virus particles released per cell) (39) are determinable variables. Genetic uniformity of the host cells is desirable, and today stable tissue culture strains can be obtained from single cells (39). This system enables isolation of virus progeny derived either from a single particle or from a single cell. It has found wide application in the study of genetics of numerous viruses and has permitted observation of the large plaque produced by a mutant of poliovirus referred to above.

Plaque size, as a characteristic in the poliovirus system, has been recently supplanted by a marker more readily determined, namely, the delayed appearance of plaques under agar overlay maintained in medium of low (0.55 gm/liter) bicarbonate content (see Table 7) (50). In an alkaline medium (2.75 gm/liter of bicarbonate), all strains of poliovirus tested show more or less equal efficiency of plating. In an acid medium, strains encountered in nature show similar plating efficiency, but the mutants, which show together with some other characteristics a decreased neuropathogenicity for rhesus monkeys, grow at a markedly delayed rate as compared with the rate in the alkaline medium; this character has been used effectively in the study of the rate of mutation of polioviruses (19) and will be discussed briefly later.

Strains with increased heat resistance have been reported for Type II (45) and

TABLE 6

DISTINGUISHING CHARACTERS OF 23 STRAINS OF POX VIRUSES*

	Ratio of strains showing following properties										
Lesion rabbit skin	Pocks										
	White	Red	Small	Large	High Virus	Low Virus	Virulence Mice	Virulence Rabbit	HA Prod.	Heat resistance High	Heat resistance Low
Small nodule	12/13	1/13	3/13	1/13	10/13	3/13	3/13	3/13	9/13	11/13	2/13
Large lesion	1/10	9/10	2/10	8/10	8/10	2/10	7/10	6/10	7/10	10/10	

* After Fenner (20).
HA = Hemagglutinin production.

TABLE 7

GENETIC MARKERS OF POLIOMYELITIS VIRUSES USED IN THE STUDY OF MUTANTS

1. Plaque size on monolayer of monkey kidney
2. Plating efficiency under agar of low bicarbonate content
3. Heat resistance
4. Growth in tissue cultures maintained at 30° C.
5. Relation to presence of cystine in tissue culture medium
6. Inhibition by normal bovine serum

Type I (17) viruses. Another character of polioviruses, determined for strains representing the three types, is the capacity to grow in tissue culture cells maintained at 30° C., or even at 23° C. (15). The observation that the optimal cytopathogenic effect of polioviruses is dependent on the presence of cystine (Chart 1) in monkey kidney tissue cultures, led to the isolation of variants which do not require cystine for cytopathogenicity and which, furthermore, have their growth inhibited by high concentrations of cystine in the tissue culture medium (14). This is shown in Chart 2, which, in addition, serves as an illustration of plaques caused by single virus particles creating in the process of multiplication necrotic foci on monkey renal epithelium monolayer, as mentioned above.

Certain sera from otherwise normal cattle were found to neutralize polioviruses. This inhibiting effect, apparently unrelated to specific neutralization, permitted selection of strains which, in contrast to those encountered in nature, became resistant to inactivation by the bovine serum inhibitor (48).

As mentioned previously, to enumerate characters of different viruses is beyond the scope of this presentation. It should suffice to say that these markers permit us a deeper approach to the study of one of the most important and intriguing aspects of virus behavior, i.e., of mutation.

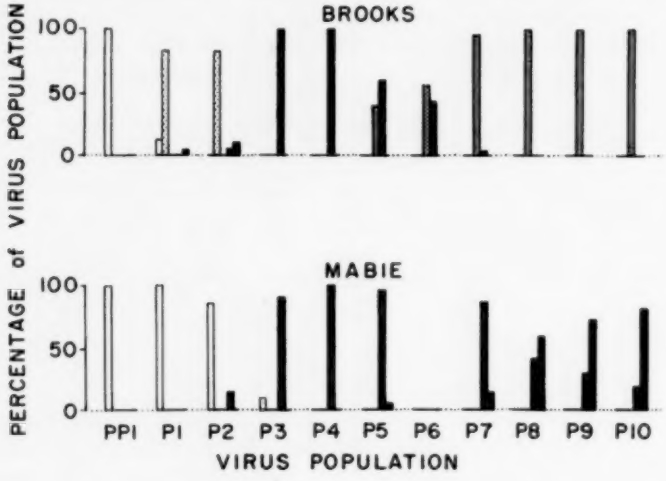


CHART 1

Virus Symbol	Response to Cystine: Requirement for	Response to Cystine: Inhibition by	Virus Symbol	Response to Cystine: Requirement for	Response to Cystine: Inhibition by
+cr	++	0	cr^si	0	+
cr^p	+	0	cr^i	0	++
cr	0	0			

After Dubes and Chapin (14).

Truth fails not, but her outward forms that bear
The longest date do melt like frosty rime

That in the morning whitened hill and plain
And is no more.
—WORDSWORTH, "Mutability," *The Weekend Book* (London: Nonsuch Press, 1955)

MUTATION, VARIATION AND ATTENUATION

Conforming to the pattern established in the preceding chapters, no attempt will be made to present an all-encompassing review of recent investigations on mutations in the field of animal viruses. Instead, examples of variation and attenuation of poliovirus will be given, since they represent a continuing challenge to investigators to correlate the readily determined characters of the virus with the elusive character of attenuation.

The early attempts to modify the disease-producing character of poliovirus followed the "historic" pattern, i.e., of adaptation to a hitherto seemingly unsusceptible host and then waiting for the grace of God to take the teeth out of the virus. This author was one of the early culprits in this scheme, and obtained strains of Type I and Type II which grew in brains of laboratory rodents (33, 34) and subsequently were tested for their relative avirulence in rhesus monkeys when injected directly into the brain. The rodent-adapted strains of poliovirus were fed to human subjects who supported the multiplication of the virus in the intestinal tract and developed antibodies, without any signs of illness whatsoever.

The advent of tissue culture techniques enabled more efficient and better controlled methods of study of virus variation. One of the methods, already of some historical value today, consisted of rapid passages of virulent virus population of high concentration in monkey kidney tissue culture tubes, or in bottles (40). The presumptive variants were then selected through limit-dilution passages and tested again in monkeys. Discovery of the plaque assay technique for poliovirus has permitted a more exact search for apathogenic strains of poliovirus. Progenies of single clones were obtained, studied for their neuropathogenicity, and the procedure of cloning was repeated until a clone with lesser pathogenicity was encountered. It involved the use of hundreds of rhesus and cynomolgus monkeys, and when the question of pathogenicity for intracerebrally injected monkeys was nearing solution, it was found that by the intraspinal route of inoculation virulent particles of the virus could still be detected.

Certain interesting facts were uncovered in the course of these investigations, e.g., that the virulence for the laboratory rodents had no relation to the neuropathogenicity for monkeys; that strains still pathogenic for monkeys were innocuous for chimpanzees injected either intracerebrally or intraspinally (41); that multiplication of virus in the human intestinal tract was unrelated to the action of the virus on monkey neurons; that strains isolated from healthy children varied widely in their pathogenicity for monkeys.

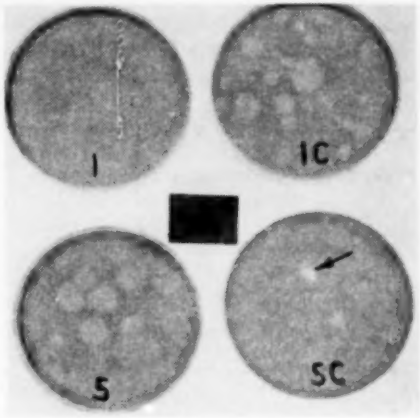


CHART 2.—Plaques formed by a cystine-requiring strain of poliovirus (Akron) and by a cystine-inhibited mutant of this strain.

1 = cystine-requiring virus in cystine-deficient medium.
1 C = cystine-requiring virus in medium with added cystine.
5 = cystine-inhibited mutant virus in cystine-deficient medium.
5 C = cystine-inhibited mutant virus in medium with added cystine.
After Dubes and Chapin (14).

Many of the strains, as mentioned above, have been tested in man and were found to multiply in the intestinal tract and to produce immunity. One notable exception was a strain with a rather interesting history of growth in the developing chick embryo (30). This Type II strain had a poor infectivity ratio for the human intestinal tract. Attempts were made to analyze the virus population of this strain and to determine whether the progenies were of uniform character. As shown in Chart 3, five clones were obtained from this strain after it was passed through the human intestinal tract. Four

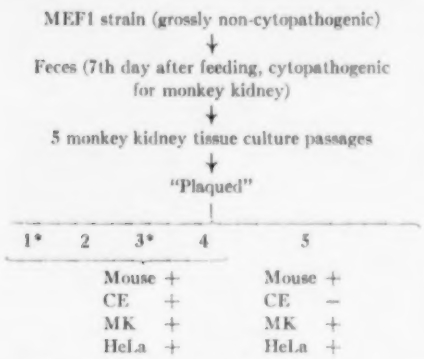


CHART 3.—Jack. Infant fed chick embryo adapted

clones grew on monkey kidney, in mice, and in chick embryo; one failed to grow in chick embryo, which indicated a mixed population of the virus, at least in relation to one determined character. The uniform inability to infect the human intestinal tract persisted as characteristic of all progenies.

It was a paradox that this relatively crude, although interesting, wrestling with the problem of apathogenicity of a virus remained for a long time a phenomenon in itself, without any associated expressions of physiologic characteristics applicable in more exact and more quantitative determinations. The crudeness of the testing system, and the fear of reversion to virulence after passage through the human intestinal tract, prompted several investigators to devise methods which would elicit markers of definite character. Availability of these markers, summarized in Table 7, permitted a study of associations with apathogenicity. This can best be illustrated by citing Dulbecco's and Vogt's (19) correlation of the mutants, characterized by delayed growth in acid medium, with monkey virulence. The ratio of plaque-forming units per ml. of a given virus on a given day in acid medium to the maximal titer in alkaline medium, given the code eop-ac (efficiency of plating [under] acid agar), has been correlated with neuropathogenicity (19). Figures for eop-ac ratio of 2×10^{-1} to 9×10^{-3} were obtained for virulent virus designated as *d*+ "wild" variants. Of the ten relatively nonpathogenic strains, the eop-ac for nine varied from 1×10^{-4} to 4×10^{-6}, and the strains have been designated as "attenuated" *d* variants. Although this eop-ac ratio seems to be closely connected with the lack of neuropathogenicity, one attenuated strain showed wild-type eop-ac ratio.

Detailed analyses of the mild *d* lines disclosed (19) that these lines give rise to mutants similar to the wild-type virus. Some of these mutants called reverse mutants arose during growth of virus in a liquid medium and are seen as a few early-appearing plaques encountered on acid-agar on the fourth day of the inoculation of the mild *d* lines. On the other hand, some mutants may produce plaques on acid agar if the *d* lines are incubated for longer periods of time (6–7 days).

Plaques harvested from acid agar plates seeded with various mild *d* lines were then analyzed for the pattern of reverse mutability. The results shown by Dulbecco and Vogt (19) for two types of poliovirus are copied on Table 8. It may be observed that, in the case of Type I virus, the mild *d* mutant (LS-c-Reed 13 ab KP 2) reached an eop-ac ratio of 10^{-4} on the sixth day. This line reverted to a mutant with the same eop-ac as the wild *d*+ type of Mahoney virus. The Type III mild *d* line had a lower value

of 4×10^{-6} for eop-ac as compared with Type I virus. However, it reverted to the type having still lower eop-ac than the wild *d*+ Leon virus. The reversion of Type I virus to a wild mutant may have occurred in a single step; the reversion of Type III may need successive steps; the mutation frequency was found to be $2\text{–}3 \times 10^{-5}$ per particle per duplication. Paradoxically, in contrast to the tissue culture experiments, the "reversion" to lines more neuropathogenic for monkeys after one passage of the attenuated *d* lines through human intestinal tract occurs more frequently in Type III infection than in Type I (40, 41). This in itself may only indicate that attempts to compare a delicate quantitative genetic analysis, such as this, with a brutal blind inoculation of the same material into the spinal cord of a monkey—when pathways of infection, cycles of multiplication, etc., are entirely unknown—is of little scientific import. One may facetiously compare such a procedure with one which has never been performed and at which, no doubt, all phage-workers would throw up their hands in horror: for instance, inject bacteriophage into a cow and correlate the bovine infection (if any) with detailed genetic analysis of the phage particles forming the inoculum.

Since monkeys are of heterogenous stock, and animal virologists notoriously disregard genetics of the host, a fact which will be dealt with briefly later, one may present an additional argument against this type of test for attenuation, an argument which in the end may sound rather mildly absurd. It runs, or rather stumbles, somewhat like this: the single monkey which will become paralyzed out of ten, or hundreds, injected with the "attenuated" variant of poliovirus may have been genetically predisposed (his own genetics) to paralysis, and this fact should not have been interpreted as "selection of a reverse mutant."

Regardless of how absurd this argument may be, it is quite certain that virulence of a virus for a given species is a property of which the underlying mechanism is still improperly understood, and the genetic tools available today may not be sufficiently refined to investigate this phenomenon adequately.

TABLE 8

PATTERN OF REVERSE MUTABILITY OF VARIOUS *d* LINES

TYPE	VIRUS STRAIN	LINE	EOP-AC ON THE 4th Day	5th Day	6th Day
1	Mahoney	d^+ (wild type) (KPLO)	$2-5\times10^{-1}$		
		d mutant (LS-c-REED 13ab KP2)			10^{-4}
		1st reverse mutants (p11, p13)	$7-8\times10^{-3}$	$1-2\times10^{-2}$	
		2d reverse mutants (p15, p16)	$4-5\times10^{-2}$	1×10^{-1}	
3	Leon	d^+ (wild type) (KP3)	$1-2\times10^{-1}$		
		d mutant ($12a_1b$)			4×10^{-6}
		1st reverse mutants (p1, p2, p3)			$4-5\times10^{-4}$
		2d reverse mutants (p7, 8)			
		3d reverse mutants (p9, p12)	$3\times10^{-3}-5\times10^{-2}$		
		4th reverse mutants (p13, p17)	$3\times10^{-2}-2\times10^{-1}$		

After Dulbecco and Vogt (19).

> Being divided, set out from colour,
> Disjunct in mid darkness
> Grazeth the light, one moving
> by the other.
> —EZRA POUND, *Canto XXXVI*

RECOMBINATIONS

Virologists, even those with a background of genetics, are only human. They like to put two things together; and the temptation is even greater if they have so many different characters to play with, as is the case with markers of mammalian viruses. So two viruses were "put together" into the same host system. And the results? One may find an answer to this in an old limerick:

> There was an old man of the Nore,
> The same shape behind as before.
> They did not know where
> To offer a chair,
> So he had to sit down on the floor.

must remain stable when passaged on limited dilutions, or as progenies of single clones. Transfer of neurotropic properties from one strain of influenza A virus to another seemed to create a recombinant in the hands of Burnet (4). In the experiments done by Hirst and Gotlieb (28, 30, 31), hybridization of the influenza A strain led to the appearance of a virus with serological properties of both parental strains and an endowment with some other markers; this may have been a possible case of heterozygosis. The identity of hybrids, obtained in the latter studies, was not retained in subsequent passages. However, it should be pointed out that a mutant was obtained by

TABLE 9

DIFFERENTIATING MARKERS OF THE INFLUENZA A STRAINS MEL AND WSE

MEL	WSE
A Serology MEL	a Serology WS
B Heat-stable HA	b Heat-labile HA
C Not indicator on heating to 55° (ovomucin)	c Indicator on heating (ovomucin)
D Not indicator on heating (sheep salivary mucin)	d Indicator on heating (sheep mucin)
E Not pathogenic for chick embryo	e Hemorrhagic lesions on CA inoculation
F* Weak lesions in mice inoculated intranasally	f Highly pathogenic for mice intranasally

* In some of the other combinations used in our experiments the symbol F was used for complete absence of lesions in mice inoculated intranasally. When any confusion might arise we have indicated the weak mouse virulence of MEL with the symbol in brackets (F).
After Burnet (4).

Reluctantly, I have to return to the influenza virus, since the first studies on genetic interaction were with strains of influenza A (4). As shown in Table 9, two strains with different serological characters, and several other markers which could be detected *in vitro*, were used for hybridization. Another important difference was, for instance, the intracerebral virulence for mice of one (the NWS) of the strains. Various types of matings occurred; in some instances seemingly true recombinants were obtained. Under this term (see Table 10) is to be understood a hybrid with serological properties of one strain, i.e., neutralized by serum containing antibody, and one or preferably more markers of the other strain. Such a virus has to breed true on subsequent passages, that is, its markers

Burnet from a strain of influenza A highly virulent for mice. This mutant possessed serologic characters of WSE but had *no* virulence for mice. Originally it was thought to be stable on passages at limited dilutions. Therefore, it was surprising to find that the loss of virulence for mice was not a true phenomenon, since this seemingly stable mutant, on passages in allantoic cavity of fertile hens' eggs, "reverted" to full mouse virulence (5).

The experimental tools available today do not permit a detailed analysis of heterozygosis versus phenotypic mixing. However, double infection with two strains of poliovirus seems to fall into the last category (36). Mating of Types I and II produced virus neutralized by Type I and Type II specific sera. The double neutralizable virus "broke

off" into the parental strain immediately after the first passage in tissue culture. Phenotypic mixing has been observed also in the mating of two strains of Newcastle virus (27). In this case, however, heterozygosis may also have taken place, though again tools for detailed analysis are not yet available.

Returning to the definition of true recombinants, experiments reported by Wildy (51) for Herpes simplex virus may indicate creation of new strains through mating of parents with different markers.

One must express his admiration for the painstaking research carried out in the pock areas of different types. About 30 per cent of single-particle progenies showed markers indicating recombination, as shown in Table 12. Some of these new viruses retained stable characters on repeated passages.

Thus, putting "two things together" may result in all sorts of reactions: in the production of legitimate offspring or homozygotes with some characters of one parent and some from the other; production of short-lived bastards who may inherit the outer characteristics (coat) of one parent but which are lost promptly (phenotypic mixing); and, finally, there are the "in-between" which

TABLE 10

EXAMPLES OF RECOMBINANTS, HETEROZYGOTES, AND PHENOTYPIC MIXING

Phenomenon	Viruses for Recombination	Authors
Recombination	Influenza A (several strains)	Burnet
	Herpes (WC×HFEM)	Wildy
	Vaccinia (neuro and dermal)	Fenner
Phenotypic mixing (possible heterozygosis)	Influenza A (MEL and NWS)	Hirst and Gottlieb
	Polio (types 1 and 3)	Sprunt, Ledinko
	Newcastle virus	Granoff

TABLE 11

MARKERS OF TWO STRAINS OF VACCINIA USED IN RECOMBINATION EXPERIMENTS

		Virulence			
Type	Pock	Mouse I.C.	Rabbit Intradermal	Hemaggl.	Heat resistance
Neuro-vaccinia	Red	High	Large lesion	None	High
Dermal-vaccinia	Gray	Low	Small nodule	High	Low

After Fenner (21).

study of markers of pox viruses. There were many markers available to Fenner to choose parents for mating in the vaccinia virus group. Finally two strains were selected which were exact opposites in their biologic characters (Table 11), and a double infection of chorioallantoic membranes was accomplished. In the first experiments, chorioallantoic membrane was inoculated with large doses of two parent strains, and properties of the virus, if any, were assayed 12 hours later. Of the 95 clones (single-particle progenies) tested, four recombinants were found (Table 12). In another experimental set-up Fenner (21) injected chorioallantois with mixtures of parent strains which give rise to overlapping of

are difficult to analyze without recourse to Freud, and which fall into the category of heterozygotes.

And he said to himself as he bolted the door,
"I will not wear a similar dress any more,
Any more, any more, any more, never more!"

—*The Complete Nonsense of Edward Lear* (London: Faber & Faber, 1947)

ARE GENETIC MARKERS OF INTACT VIRUS STILL NEEDED?

It has been suspected for some time that the nucleic acid in the nucleoprotein complexes, which we call viruses, plays the prominent role as transmitter of genetic in-

formation. To prove this point beyond any possibility of doubt, it became necessary to divest the virus of its protein dress and view it in its naked but still recognizable form. The act of undressing may be a pleasurable one or not, depending on the circumstances, but it is difficult to put one's self in the position of a virus in the process of shedding its clothing. During the past year, Colter *et al.* (10, 11) were able to undress three mammalian viruses by means of a bath in water-saturated phenol. Removal of phenol by ether extraction completed the undressing, and the virus stood in all its naked, infectious splendor. In Table 13 are shown examples of five viruses which were subjected to such undressing and which, when naked, consisted of ribonucleic acid deprived of its protein coat, but still infectious.

Since the technique of phenol extraction is today quite a simple laboratory procedure, it is possible that, while this review is being read, other viral agents have been similarly undressed. This, however, is not true of all viruses. Attempts have been made in various laboratories to isolate an infectious RNA from viruses which belong to the "genetically belabored" myxo-group, such as fowl plague and influenza. Thus far, for some unexplained reason, these viruses have not been amenable to the wiles of the "undressers."

TABLE 12

EXAMPLES OF THE STABLE RECOMBINANTS OBTAINED IN EXPERIMENTS WITH RABBITPOX AND DERMAL VACCINIA VIRUSES

Italics indicate the features in which the strain in question differs from the parent dermal vaccinia strain.

Strain	CAM	MV	RS	HA	HA
Rabbitpox parent	R	h	PC	*0*	h
Dermal vaccinia parent	W	l	N	h	l
R1-1	W	l	N	*0*	l
R1-3	W	*int.**	*int.*	h	l
R1-4	W	l	N	h	*h*
R2-3	W	*int.*	*int.*	h	*h*

* int. = intermediate between the behavior of the two parent strains.

CAM = pock appearance
MV = mouse virulence
R = hemorrhagic
W = opaque white
RS = intradermal inoculation of rabbits
HA = hemagglutinin production
h = high
l = low
HR = heat resistance
PC = purple center
N = red nodule

After Fenner (21).

TABLE 13

INFECTIOUS RNA OF MAMMALIAN VIRUSES

Virus	Authors
West Nile	Colter *et al.*
Mengo	Colter *et al.*
Polio	Colter *et al.* Alexander *et al.*
Eastern equine encephalitis	Wecker and Schäfer
Semliki forest	Chang

The infectivity of the five ribonucleic acids did not exceed 0.1 per cent of that of the viruses from which they were isolated (9). Properties of the naked RNA and the intact virus are summarized in Table 14. In contrast to the intact virus, the infectivity of RNA was abolished by treatment with ribonuclease and 6 hours of incubation at 37° C. Treatment with 1 M NaCl for 12–16 hours markedly decreased the infectivity of the intact virus, but it had little or no effect on the infectious RNA. As was to be expected, the intact virus particles sedimented more rapidly than the infectious component in the RNA preparation (9).

The action of serum requires more explanation. In experiments conducted by Schramm and Gierer (42) with plant viruses, immune serum which neutralized the intact

tobacco mosaic virus (TMV) had no effect on the infectious, naked RNA component, although both normal and immune serum showed a certain amount of inactivating capacity. Unfortunately, this was much more accentuated in the case of animal virus RNA, and, therefore, the elegant experiments showing lack of effect of immune serum in the absence of protein dress are as yet inconclusive.

Even if the fact is taken into consideration that some viruses are reluctant to shed their protein dress, and may remain so forever, the availability of several infectious ribonucleic acids isolated from different viral preparations may be of enormous importance for the genetics of animal viruses. The study of configuration of bases in the RNA preparation may already show differences between various viruses. This may be wishful scientific optimism, but it should be remembered that Ada and Perry (1) have shown that ribonucleic acid (in an impure preparation) of influenza A virus had a characteristically different pattern of bases from that of influenza B. The availability in the future of such chemically defined characters could make the other genetic markers of viruses discussed above look rather obsolete.

The availability of an infectious RNA may also help to elucidate, at least partially, the comparative importance of naked RNA *vis-à-vis* its nucleoprotein complex in the carrying out of biological activity. The question arises whether the nucleic acid is the sole genetic marker, the protein part acting only as a "public relations department" (38). To discuss the problem intelligently one has to digress for a moment from the fauna of animal viruses and invade again the flora of plant viruses. The naked RNA of tobacco mosaic virus is infectious. However, when mixed with the inactive protein component of TMV the infectivity is increased. This, and other arguments, including that of electron micrograph preparations, would seem to indicate that, through recombination of RNA and protein, a reconstituted virus was formed (24). Thus, at least partial evidence was obtained that the two components of TMV virus recombined in a specific manner. But, which component was the master of biological activity?

In some rather complicated experiments Fraenkel-Conrat and Singer (22, 23) have reconstituted viruses through recombination of RNA and inactive viral protein obtained from two or more different strains of TMV. These reconstituted nucleoproteins were tested for infectivity in plants, and the resulting progenies were chemically and serologically analyzed. The results indicated that the protein of the progeny resembled, or was identical with, that of the protein from which the infectious RNA had been obtained and seemingly was unrelated to the protein which was used for the reconstitution of the inoculum (22, 23). Although an attempt was made to prove that in certain instances the protein dress may influence the character of nucleic acid (52), in essence, we may accept the possibility that the RNA of the TMV virus is the master of genetic activity. The public relations role of protein has also been proved through the observation that immune serums were specific for protein components (23).

TABLE 14

COMPARISON OF CHARACTERISTICS OF INTACT VIRUS AND VIRUS RNA

Test	Intact virus	Virus RNA
Ribonuclease	No effect	Inactivation
6 hours at 37 C.	No effect	Inactivation
1 M NaCl	Decreased infectivity	No effect
Sedimentation velocity	Rapid	Slow
Immune serum	Neutralization	?*

* See text for discussion on "inactivation" by normal serum.

In the case of animal virus RNA it is not possible as yet to conduct the same sort of experiments as were done with TMV because of impurity of animal virus preparations. However, the role of RNA could be ascertained if exposure of the test system—hitherto found resistant to intact virus infections—were attempted. Would such a system be susceptible to the naked RNA, in contrast to the intact virus? A parallel perhaps may be drawn with the studies of bacteriophage DNA (44, 52).

The T2 phage disrupted by osmotic shock, and containing infectious DNA, cannot infect bacteria which are susceptible to the intact phage. However, the shockates will infect protoplast not only of susceptible strains but also of some resistant strains (44). In the introduction mention had been made that in this one aspect the animal virologists have advanced beyond the phage-geneticists; their infectious RNA preparations consist of protein-free nucleic acid, whereas the phage DNA may still be protein-bound (25) but probably not for very long in terms of chronology of scientific observations.

TABLE 15

EXTREMES OF HOST SPECIFICITY

Virus	Host Range
Rabies	All homeothermal animals
Influenza	Man, ferret, mouse, chick embryo
Adeno	Man and tissue culture
AKR mouse leukemia	Z subline of C3H strain of mice*

* The susceptibility of the AKR strain cannot be easily verified because of the incidence of spontaneous leukemia.

In the forthcoming (I hope) studies on the role of RNA as determinant of genetic constitution of the particles, recombination experiments between two different RNA may be even more exact and more exciting. Many other discoveries may be made by using infectious mammalian RNA as an experimental source, and a new era of animal virus genetics may be in the offing. The naked virus may yet become the dressed, in any style it may desire.

There lived an old man in the Kingdom of Tess,
Who invented a purely original dress;
And when it was perfectly made and complete
He opened the door and walked in the
street . . .

The last class of illusions are those which cannot be discovered within one person's experience, except through the discovery of discrepancies with the experiences of others.—BERTRAND RUSSELL, *Mysticism and Logic* (Doubleday, W.V., 1951)

IS THE HOST NECESSARY?

It would be unwise to leave the audience with illusions of starry-eyed optimism, and it should be brought back to earth with a question: After all that has been said, is the host still necessary? Obviously yes, not only to provide the viruses with rich soil for their propagation, mutation, and any other tricks of their trade to enable their survival, but also to furnish the workers in the field a source for receiving grants for their studies. If the host is necessary, should it then be disregarded? How much can the host contribute in its intact (and not in tissue culture) form to our knowledge of virus genetics? The answer, again, is quite a lot.

The last table (number 15) summarizes the extremes of host range for viruses. Universal susceptibility to rabies virus may be sharply contrasted with the extreme specific susceptibility of inbred sublines of a highly inbred strain of mice to a leukemia virus. Despite the wide spectrum of animals susceptible to rabies infection, why do tissue culture explants stubbornly resist the infection with the same virus, in marked contrast to the adeno-viruses which grow with equal ease in man and in his tissues *ex corpora?* Is the disregard for the genetic origin of the host justifiable? Are kidney cell explants obtained from different monkeys at various time intervals similar in their susceptibility to virus infection, though obtained from a heterozygous breed? Should "adaptation" of a virus to a Swiss mouse lead to shouts of joy, or rather to expression of sorrow that a genetically inbred strain of mice had not

been retained as host in lieu of a heterozygous stock. What is adaptation, virulence, attenuation?

Answers to these questions are either unavailable or within the realm of delightfully speculative hypotheses, indicating that, wise as we may seem to be, there still remains much to be learned.

The art of quotation requires more delicacy in the practice than those conceive who can see nothing more in a quotation than an extract.—DISRAELI

ENVOI—SCIENCE AND MAGIC

Sir James G. Frazer has this to say in *The Golden Bough* (26):

But while science has this much in common with magic that both rest on a faith in order as the underlying principle of all things, readers of this work will hardly need to be reminded that the order presupposed by magic differs widely from that which forms the basis of science.

And yet later on he qualified this:

We must remember that at bottom the generalizations of science, or, in common parlance, the laws of nature are merely hypotheses devised to explain that ever-shifting phantasmagoria of thought which we dignify with the high-sounding names of the world and the Universe. In the last analysis magic, religion, and science are *nothing but theories of thought.* (Italics are mine.)

It must by now be quite clear that "faith in order as the underlying principle" of genetics of animal viruses is based as much on "theories of thought" as on "patient, exact observation of the phenomena themselves." If this faith is somewhat wobbly at times, it is because of the existing confusion which prevents us from seeing "order as the underlying principle" in the classification of the hosts to the viruses—that is, animals.

Robert Graves (29) quotes a story which he calls "ancient." An old lady traveling by train from London to Edinburgh was taking a pet tortoise in a basket and wanted to know whether she should buy a dog-ticket for it, as one is required to do in England for cats because cats officially count as dogs. "No," said the ticket inspector, "no mum. Cats is dogs, and rabbits is dogs, and dogs is dogs; and squirrels in cages is parrots, but this here turkle is a hinsect. We won't charge you nothing, mum!"

REFERENCES

1. ADA, G. L., and PERRY, B. Specific Differences in Nucleic Acids from A and B Strains of Influenza Virus. Nature, **175**:854–55, 1955.
2. ANDREWES, C. H., and HORSTMANN, D. M. The Susceptibility of Viruses to Ethyl Ether. J. Gen. Microbiol., **3**:290–97, 1949.
3. BLANK, H., and RAKE, G. Viral and Rickettsial Diseases of the Skin, Eye and Mucous Membranes of Man. Boston: Little, Brown & Co., 1955.
4. BURNET, F. M. Genetics of Animal Viruses. Handbuch der Virusforschung, **4**:1–26. Wien: Springer-Verlag, 1958.
5. ———. Genetics of Influenza Virus. Australian J. Sc., **20**:74–75, 1957.
6. BURNET, F. M., and BULL, D. R. Changes in Influenza Virus Associated with Adaptation to Passage in Chick Embryos. Australian J. Exper. Biol. Med. Sc., **21**:55–69. 1943.
7. BURNET, F. M., and STONE, J. D. Further Studies on O-D Changes in Influenza Virus A. Australian J. Exper. Biol. Med. Sc., **23**:151–60, 1945.
8. CASALS, J. Viruses: The Versatile Parasites. I. The Arthropod-Borne Group of Animal Viruses. Trans. New York Acad. Sc., Ser. 2, **19**:219–35, 1957.
9. COLTER, J. S. Nucleic Acid as the Carrier of Viral Activity. Progress M. Virol., **1**:1–35, 1958.
10. COLTER, J. S.; BIRD, H. H.; and BROWN, R. A. Infectivity of Ribonucleic Acid from Ehrlich Ascites Tumor Cells Infected with Mengo Encephalitis. Nature, **179**:859–60, 1957.
11. COLTER, J. S.; BIRD, H. H.; MOYER, A. W.; and BROWN, R. A. Infectivity of Ribonucleic Acid Isolated from Virus-Infected Tissues. Virology, **4**:522–32, 1957.
12. DOWNIE, A. W. Jenner's Cowpox Inoculation (Jenner Memorial Lecture). Brit. M. J., **2**:251–56, 1951.

13. DOWNIE, A. W., and HADDOCK, D. W. Variant of Cowpox Virus; Preliminary Communication. Lancet, **1**:1049–50, 1952.
14. DUBES, G. R., and CHAPIN, M. Poliovirus Mutants with Altered Responses to Cystine. J. Gen. Microbiol., **18**:320–29, 1958.
15. DUBES, G. R., and WENNER, H. A. Virulence of Polioviruses in Relation to Variant Characteristics Distinguishable on Cells *in Vitro*. Virology, **4**:275–96, 1957.
16. DULBECCO, R. Interactions of Viruses and Animal Cells; Study of Facts and Interpretations. Physiol. Rev., **35**:301–35, 1955.
17. ———. Quantitative Aspects of Virus Growth in Cultivated Animal Cells. *In:* Ciba Symposium on The Nature of Viruses, p. 147, 1956.
18. DULBECCO, R., and VOGT, M. Biological Properties of Poliomyelitis Viruses as Studied by the Plaque Technique. Ann. New York Acad. Sc., **61**:790–800, 1955.
19. ———. Study of the Mutability of *d* Lines of Polioviruses. Virology, **5**:220–35, 1958.
20. FENNER, F. The Biological Characters of Several Strains of Vaccinia, Cowpox and Rabbit-Pox Viruses. Virology, **5**:502–29, 1958.
21. ———. Genetic Studies with Vaccinia Virus. Australian J., Sc., **20**:75–76, 1957.
22. FRAENKEL-CONRAT, H. The Role of the Nucleic Acid in the Reconstitution of Active Tobacco Mosaic Virus. J. Am. Chem. Soc., **78**:882–83, 1956.
23. FRAENKEL-CONRAT, H., and SINGER, B. Virus Reconstitution. II. Combination of Protein and Nucleic Acid from Different Strains. Biochim. et Biophys. Acta, **24**: 540–48, 1957.
24. FRAENKEL-CONRAT, H., and WILLIAMS, R. C. Reconstitution of Active Tobacco Mosaic Virus from Its Inactive Protein and Nucleic Acid Components. Proc. Nat. Acad. Sc., **41**:690–98, 1955.
25 FRASER D.; MAHLER, H. R.; SHUG, A. L.; and THOMAS, C. A., JR. The Infection of Sub-Cellular *Escherichia coli*, Strain B, with a DNA Preparation from T2 Bacteriophage. Proc. Nat. Acad. Sc., **43**:939–47, 1957.
26. FRAZER, SIR J. G. The Golden Bough. New York: The MacMillan Co., 1958.
27. GRANOFF, A. (to be published).
28. GOTLIEB, T., and HIRST, G. K. Experimental Production of Combination Forms of Virus; Formation of Doubly Antigenic Particles from Influenza A and B Virus and Study of Ability of Individual Particles of X Virus To Yield Two Separate Strains. J. Exper. Med., **99**:307–20, 1954.
29. GRAVES, R. Five Pens in Hand. New York: Doubleday & Co., 1958.
30. HIRST, G. K., and GOTLIEB, T. Experimental Production of Combination Forms of Virus; Study of Serial Passage in Allantoic Sac of Agents That Combine Antigens of Two Distinct Influenza Strains. J. Exper. Med., **98**:53–70, 1953.
31. ———. The Experimental Production of Combination Forms of Virus. V. Alterations in the Virulence of Neurotropic Influenza Virus as a Result of Mixed Infection. Virology, **1**:221–35, 1955.
32. KOPROWSKI, H. Biological Modification of Rabies Virus as a Result of Its Adaptation to Chicks and Developing Chick Embryos. Bull. World Health Organ., **10**:709–24, 1954.
33. KOPROWSKI, H.; JERVIS, G. A.; and NORTON, T. W. Immune Responses in Human Volunteers upon Oral Administration of a Rodent-Adapted Strain of Poliomyelitis Virus. Am. J. Hyg., **55**:108–26, 1952.
34. KOPROWSKI, H.; NORTON, T. W.; JERVIS, G. A.; NELSON, T. L.; CHADWICK, D. L.; NELSEN, D. J.; and MEYER, K. F. Clinical Investigations on Attenuated Strains of Poliomyelitis Virus. Use as a Method of Immunization of Children with Living Virus. J.A.M.A., **160**:954–66, 1956.
35. LEDINGHAM, J. C. G. Comparative Study of Clinically Allied Viruses: Some Unsolved Problems of Edward Jenner: President's Address. Proc. Roy. Soc. Med., **29**:73–82, 1935.
36. LEDINKO, N. (to be published).
37. MOGABGAB, W. J.; GREEN, I. J.; and DIERKHISING, O. C. Primary Isolation and Propagation of Influenza Virus in Cultures of Human Embryonic Renal Tissue. Science, **120**:320–21, 1954.
38. MUDD, S. (personal communication).
39. PUCK, T. T., and MARCUS, P. I. A Rapid Method for Viable Cell Titration and Clone Production with HeLa Cells in Tissue Culture. Proc. Nat. Acad. Sc., **41**:432–37, 1955.
40. SABIN, A. B. Present Status of Attenuated Live-Virus Poliomyelitis Vaccine. J.A.M.A., **162**:1589–96, 1956.
41. ———. Properties of Attenuated Polioviruses and Their Behavior in Human Beings. *In:* Cellular Biology, Nucleic Acids

and Viruses, Special Publ. New York Acad. Sci., **5**:113–27, 1957.

42. SCHRAMM, G., and GIERER, A. Investigations on the Ribonucleic Acid of Tobacco Mosaic Virus. *In:* Cellular Biology, Nucleic Acids and Viruses, Special Publ. New York Acad. Sc., **5**:229–34, 1957.
43. SMITH, W. Action of Bile Salts on Viruses. J. Path. & Bact., **48**:557–71, 1939.
44. SPIZIZEN, J. Infection of Protoplasts by Disrupted T2 Virus. Proc. Nat. Acad. Sc., **43**:694–701, 1957.
45. STANLEY, N. F.; DORMAN, D. H.; PONSFORD, J.; and LARKIN, M. Isolation of a Heat-Resistant Variant of Polio-Virus. Nature, **178**:413–14, 1956.
46. STRODE, G. K. (ed.). Yellow Fever. New York: McGraw-Hill Book Co., 1951.
47. SULKIN, S. F., and ZARAFONETIS, C. Influence of Anesthesia on Experimental Neurotropic Virus Infections; *in vitro* Studies with Viruses of Western and Eastern Equine Encephalomyelitis, St. Louis Encephalitis, Poliomyelitis (Lansing) and Rabies. J. Exper. Med., **85**:559–69, 1947.
48. TAKEMORI, N.; NOMURA, S.; NAKANO, M.; MORIOKA, Y.; HENMI, M.; and KITAOKA, M. Mutation of Polioviruses to Resistance to Neutralizing Substances in Normal Bovine Sera. Virology, **5**:30–55, 1958.
49. THEILER, M. Action of Sodium Desoxycholate on Arthropod-Borne Viruses. Proc. Soc. Exper. Biol. & Med., **96**:380–82, 1957.
50. VOGT, M.; DULBECCO, R.; and WENNER, H. A. Mutants of Poliomyelitis Viruses with Reduced Efficiency of Plating in Acid Medium and Reduced Neuropathogenicity. Virology, **4**:141–55, 1957.
51. WILDY, P. Recombination with Herpes Simplex Virus. J. Gen. Microbiol., **13**:346–60, 1955.
52. WONG, T. Y., and COMMONER, B. The Formation of Infectious Nucleoprotein from Tobacco Mosaic Virus Protein and Tobacco Leaf DNA. Proc. Nat. Acad. Sc., **42**:831–41, 1956.

Discussion*

GEORGE K. HIRST
Public Health Research Inst., New York, N.Y.

The impact of genetics on the virus field, both bacterial and human, can best be appreciated if one tries to recall the confused state of knowledge concerning the nature of viruses as recently as 10 years ago. This confusion was rapidly resolved after Hershey and his co-workers demonstrated that viruses were organisms with a genetic apparatus very similar to that of higher forms. The demonstration that bacterial viruses had a chromosome made of DNA and bearing genes in linear array, however, did much more than unite viruses with other biological species. Through the study of bacterial viruses by genetic methods, it has been possible to get an increasingly clear picture of how viruses multiply and reproduce themselves within the host cell. We now have clues about the evolutionary relationship of the virus and the host cell. In addition, bacterial virology is making contributions to classical genetics and has been especially valuable in extending the biochemical attack on genetic and biosynthetic problems. The relationships between bacteria and their viruses are such that it is now impossible to study either virus or host cell without an intimate knowledge of the other. One of the effects of the study of genetics of bacteriophage has been the stimulation of similar studies in the animal virus field.

Animal viruses may be divided into two large groups based on the type of nucleic acid which they contain. Some are made up principally of DNA, and some contain RNA. So far, no virus contains both. Both kinds of virus give rise to mutations, and, with the use of these as markers, recombination experiments can be carried out. Among the DNA viruses, a few preliminary studies have shown that genetic recombination can take place between different strains of vaccinia virus and of herpes simplex virus. Among the RNA-containing viruses, influenza, Newcastle disease virus, and human poliomyelitis virus are the ones which have been principally studied. Thus far, recombination has been demonstrated only with influenza virus. Most of the genetic work with animal viruses has been done with the relatively clumsy tools which have been available for some time. No definitive studies have been done with any animal virus by the modern quantitative virological techniques of plaque formation and infection of single cells. It is not known, for example, for any animal virus, whether the genes for different characters are carried in a linear order. The problems of greatest interest lie in the future.

An example of the type of information which is already existent in studies of influenza and polioviruses concerns phenotypic mixing. When the distribution of genetic markers is studied following the infection of a cell with two different distinguishable virus strains, the progeny show certain changes in the phenotype, either in type specificity or in the nature of the hemagglutinin, and this alteration can be demonstrated to be non-inheritable in character. A mixed infection with type A and type B influenza viruses yields particles that have both A and B antigens on the surface of such particles. The situation seems to be that the genetic material, presumably the nucleic

* Following paper by Dr. Koprowski.

acid, from the two virus strains multiplies independently and specifically within the same host cell but that the coating of this material by virus-induced protein or other substance is a non-specific process and the nucleic acid from one strain may be coated with material characteristic of another strain. This is called phenotypic mixing and has been known for a long time with bacterial viruses.

With animal viruses, especially those of the myxo group, there is now independent evidence based on morphological findings which is consistent with this interpretation of phenotypic mixing (Breitenfeld and Schäfer, *Virology*, **4**:328–45, 1957). When fowl plague virus, which is closely related to influenza A, infects an isolated cell (such as a chick fibroblast), studies with fluorescent antibodies reveal that an early consequence of this infection is the appearance in the nucleus of a nucleic acid or nucleoprotein which is characteristic of the virus and which does not appear initially in the cytoplasm. This nucleic acid or nucleoprotein cannot be detected by the same reagents in the intact virus particle, so it presumably is in some naked or uncovered state in the cell nucleus.

At a later stage of infection, the presence of coating material can be detected in the cytoplasm, and this coating material which appears diffusely throughout the entire cytoplasmic portion of the cell does not occur in the nucleus. At a still later stage, the demonstration of finished, coated particles can be seen in the cell membrane of the host cell by electron microscopy. Careful study of the infected cell shows that this coating takes place only in the cell wall. From this sort of evidence, it becomes readily understandable how coating occurs in a non-specific fashion.

In attempting to assess the importance of genetic studies on animal viruses, stress should be laid on their possible importance in those conditions where there is an intimate, long, and at present obscure relationship between the virus and the host cell. Such conditions are found pre-eminently in the relationship between tumor-inducing viruses and their host cells and in latent virus conditions. Alternatively, the genetics of mammalian viruses will probably be of little consequence in combatting infectious diseases where the virus has a purely cytopathogenic effect. The virus etiology of cancer is receiving increasing attention and offers possibilities of studying the mechanism of cancer inception in normal cells. It is not inconceivable that, with the continued expansion of the field of virology and the spread of the revolutionary techniques which have recently been introduced (especially in cell culture), we will witness a parallel development in the study of animal viruses and the study of somatic host cells in which each will complement the other.

Bacterial Genetics and Infectious Disease

B. A. D. STOCKER
Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1., England

Bacteriologists from Pasteur onward have been much concerned with "bacterial variation" and "dissociation," i.e., with problems of the genetics of bacteria, and with their relation to pathogenicity. In recent years the use of bacteria as experimental material for genetical research has led to discoveries of great importance to genetics and biology generally—e.g., to the identification of deoxyribonucleic acid as the material basis of the gene. The application to bacteriology of concepts developed in the study of the genetics of higher organisms has greatly increased our understanding of inheritance in bacteria. We now appreciate that in bacteria, as in higher organisms, it is essential to distinguish the phenotype, i.e., the actual observable characters of the individual organism, and the genotype, i.e., the sum of all the heritable characters, either actually expressed or capable of expression in a suitable environment; and that the phenotype of a bacterium is determined both by its genotype, or hereditary constitution, and by the environment in which it grows (in some cases the environment of the immediately preceding generations also has some influence). Bacteria divide by binary fission, and the genotype of the two daughter cells, and of their descendants, is identical with that of the parent bacterium, except when a change is caused either by *genetic recombination* or by *mutation*. These terms are here used in a wide sense—"recombination" to cover all cases in which a bacterium interacts with another organism (bacterium or bacteriophage) and in consequence produces progeny having an altered hereditary constitution; and "mutation" to describe all changes in hereditary constitution in which no second organism is involved.

In bacteria a great variety of characters are subject to change by mutation, different mutations occurring at characteristic rates, from as high as one in a hundred to as low as one in ten thousand million per bacterium per generation; the rate may be increased by mutagenic treatments, e.g., ultraviolet irradiation, or decreased by other treatments. In *Escherichia coli*, a species which "mates," genetical analysis has shown that many different mutants behave as if their mutant character resulted from a change at a localized spot in a linearly arranged group of genes (linkage map). In other genera, e.g., Pneumococcus, in which DNA transformations can be effected, it is found that a mutation in a bacterium is associated with a corresponding change in the transforming properties of the DNA extractable from the bacterium. For instance, DNA extracted from a penicillin-resistant mutant strain of pneumococcus will transform other pneumococci to heritable resistance, whereas DNA extracted from the parent sensitive strain will not. We infer that a bacterial mutation consists of a localized change in the genetically active DNA of the bacterium, which is arranged linearly in a linkage group or chromosome. In higher organisms the genotype may change in ways other than by mutation of a chromosomal gene; for instance, the number of chromosomes per nucleus may be doubled, or the organisms may lose some "self-replicating" cytoplasmic particle which cannot be synthesized *de novo*, e.g.,

a chloroplast. Some such changes may occur in bacteria also. Mutation of a chromosomal gene seems, however, to be the most common cause of change in bacterial genotype. Since in most cases the mechanism has not been investigated, I shall for the present purpose use "mutation" in a wide sense, without necessarily implying that mutation of a chromosomal gene is involved.

In higher organisms genetic recombination results almost always from a sexual process: in bacteria a variety of different methods have been discovered The one most closely resembling recombination through a sexual process was discovered in *Escherichia coli;* in this organism two bacteria conjugate, a part or the whole of the (solitary) chromosome of one partner passes into the other partner, to form a zygote, and crossing over between the two chromosomes follows, resulting in the production of a recombinant or hybrid bacterium, deriving some of its characters from one parent strain and some from the other. The segregation of particular characters, and by inference of the genes which control them, has enabled geneticists to chart the genes on a linear linkage map, which we believe is a representation of the bacterial chromosome. In this mating process in *E. coli* any bacterial character may recombine. There is also known among coliform bacteria a process in which conjugation between cells of two different strains results in one partner's acquiring just *one* character from the other; the characters thus transmissible are the ability to produce a colicine (an antibiotic active on certain coliform bacteria) and the "F" (fertility) character, required for ability to conjugate. We do not know whether the transfer of these characters (and of no others) during "conjugation" between certain strains results from the transfer of some hypothetical self-replicating cytoplasmic particle or from the transfer of a very short piece of chromosome bearing genes controlling these characters.

In addition to these processes of recombination by means of cell conjugation, we have available in the laboratory two methods of extracting genes from the disrupted cells of one strain (the gene donor strain) and transferring them into whole cells of a second, recipient, strain, where they recombine with the chromosomal genes of the recipient bacterium. In some genera (Pneumococcus, Haemophilus, etc.) one can dissolve the cells of the donor strain and extract the genes in the form of macromolecular deoxyribonucleic acid; this DNA will penetrate into cells of the recipient strain and recombine with the genes of these cells. As a result, a proportion of the treated cells acquire some new character from the donor strain from which the DNA was extracted and transmit this character to all their descendants. The process of genetic transduction by phage discovered in Salmonella is, we believe, essentially similar, but a phage particle is needed to carry the genetic material from one bacterium to the other; free DNA is ineffective. The cells of the donor strain are lysed, by growing phage in them; each bacterium bursts, liberating some hundreds of phage particles, some of which contain fragments of the chromosome of the lysed bacterium. When such a phage particle is absorbed by a bacterium of the recipient strain, the chromosome fragment from the previous host recombines by crossing over with the chromosome of the recipient bacterium, to produce a recombinant. In both these processes, transformation by DNA and phage-mediated transduction, only a small piece of foreign genetic material enters the cell, and contributes, by crossing over, to the genetic constitution of the recombinant, which is thus a hybrid deriving nearly all its characters from one parent, the recipient strain, and only one or a few from the other parent, the lysed, donor, strain.

Only a small minority of the phage particles in a Salmonella lysate carry bacterial genes from one bacterium to another in this way. However, in some phage strains each phage particle which attacks a sensitive bacterium has a good chance of establishing itself as a stable hereditary parasite in the bacterium, so that every one of this bac-

terium's descendants inherits the potentiality of liberating phage particles; such bacteria are called lysogenic. It is believed that in a lysogenic bacterium the phage genes are attached to the bacterial chromosome and are replicated *pari-passu* with it. When a bacterium becomes parasitized in this way, it may in consequence change its character in certain respects (*phage conversion*): for instance when non-toxin-producing strains of diphtheria bacillus acquire a heritable infection with certain phages they are converted into toxin-producing strains. The distinction between the transfer of bacterial genes to a new bacterium by a phage particle and the attachment of phage genes to the bacterial chromosome is not clear in some cases; we then cannot clearly distinguish between genetic transduction and lysogenic conversion: both, however, may be treated as instances of genetic recombination in bacteria.

The hereditary constitution of a bacterial strain may therefore change by mutation, or by any of several different processes of recombination. The comparison of bacterial strains of different hereditary constitutions, obtained by mutation or recombination, has been of great importance in the experimental study of bacterial infections of man and other animals.

GENETICS OF BACTERIAL VIRULENCE

I propose first to consider the role of the genetic constitution of the bacterium in determining its virulence when administered to an experimental animal of a particular species, and to defer consideration of the more complex case of naturally occurring infective disease. I use "virulence" here to mean the ability of the administered bacteria to multiply in the host and thereby to produce a pathological process; and, in a quantitative sense, to indicate the capacity of a bacterial strain to produce an infection from a small, rather than a large, challenge dose. The question which we seek to answer is what bacterial characters (in terms of bacterial physiology and anatomy) are required for virulence when the bacteria are administered to a particular host by a particular route.

The most clear-cut answers have been obtained in respect to bacterial characters found to be essential for virulence. Long before the days of the systematic study of bacterial genetics it was noted that, for instance, all virulent strains of pneumococcus and anthrax bacillus had the capacity to form capsules (at least in the environment provided by the host) and that all virulent Salmonella strains possessed the surface antigenic component conferring "smoothness." In many such instances the gain, or loss, by mutation, of the character associated with virulence can be easily demonstrated *in vitro;* for instance, in the pneumococcus one may, by the use of anti-"S" or anti-"R" sera obtain nonencapsulated variants from encapsulated strains and vice versa (see, for instance, [12]); the role of the serum is probably only to select spontaneously occurring mutants, not to induce them. The change in virulence which is found to accompany the change in bacterial character, e.g., encapsulation, is evidence that this character is essential for virulence, not merely an accidental concomitant of virulence in naturally occurring strains. The proof is completed when one can confer the character in question on a nonvirulent strain which lacks it, by some method of genetic recombination in which it is known that only a very small fraction of the genes of the recipient strain are altered. This was first accomplished by Griffith in his original work on "type-transformation" in pneumococci (11), but the genetic aspects of the phenomenon were not well understood until the transformation of a nonvirulent nonencapsulated pneumococcus to encapsulation, and virulence, was achieved *in vitro*, by the use of deoxyribonucleic acid extracted from an encapsulated strain.

An alternative approach to this problem of what characters are essential for virulence is to start with a virulent strain and to prepare from it a series of mutants, differing from it in respect to various biochemical or other characters, and to test the

virulence of these mutants. This was done in *Salmonella typhi* by Bacon, Burrows, and Yates (2), using the mouse as test host. (*Salmonella typhi* does not normally infect mice, and indeed a very large number of bacteria must be inoculated to produce a fatal infection in this animal, so that the system investigated was a highly "artificial" one; but it seems that the results obtained are also applicable to infections more nearly resembling those which occur in nature, e.g., infections of mice by *S. typhimurium*).

Mutants of the typhoid bacillus requiring an exogenous source of purine, para-aminobenzoic acid, or aspartic acid, were found to be of greatly diminished virulence (in that a much larger number of bacteria injected intraperitoneally was required to produce a fatal infection); whereas the virulence of mutants requiring various other growth factors differed little or not at all from that of the parent strain. It was shown that the peritoneal fluid of the mouse did not contain sufficient of the relevant growth factor (purines, PABA, or aspartic acid) to support the growth of the mutants and that the mortality of mice inoculated with, for instance, the PABA-requiring strain was increased if this substance was administered to the mouse. Back-mutants, no longer requiring any special growth factor, were isolated *in vitro* from some of these nutritionally exacting strains of diminished virulence; the reverted forms were found to have regained virulence, as well as the ability to dispense with the relevant growth-factor. These experiments establish that an essential criterion for virulence is that the nutritional requirements of the bacterium shall be satisfied by the pabulum provided by the relevant tissue of the host. This seems an obvious point, but one which seems to have been overlooked in the past. It seems to me that this line may, when applied in other situations, yield results of even greater interest. In the systems thus far studied the bacteria causing an infection multiply in the extra-cellular fluids of the host; it is relatively easy to obtain samples of such material and test whether it contains sufficient of a particular metabolite to support the growth of a nutritionally exacting bacterium. The case is otherwise when we are dealing with bacteria which multiply within the cells of the host. Testing the virulence of nutritionally exacting mutants of such bacteria may perhaps provide information as to the availability of various nutrients *within* the mammalian cell. Such information is essential in the rational design of antimetabolites for use in the chemotherapy of infections by bacteria which multiply intracellularly: if PABA were present in the intercellular fluids of the mouse, sulfonamide therapy would be ineffective in this animal.

We can thus define certain characters, in particular the presence of some components at the bacterial cell surface and the capacity to grow without an external source of certain metabolites, which are necessary for virulence in particular systems. Such characters are not, however, sufficient for virulence, as is evident from the fact that many bacterial species are virulent for one mammalian host but not for another; for instance, most strains of *Salmonella typhimurium* after serial passage became highly virulent for mice, the LD_{50} by intraperitoneal injection being commonly less than ten bacteria, whereas the LD_{50} for the closely related organism *S. paratyphi B*, even after serial passage, is in the region of 10^7 bacteria. These two bacteria have identical somatic antigens and are both nutritionally nonexacting, and the reason for the difference in their mouse virulence is unknown, the characters relied upon for their differentiation, e.g. their flagellar antigens, being almost certainly not concerned in virulence. It is possible that the bacterial geneticist can help in the search for the relevant difference between the two species, for one can transfer characters, one at a time, from *S. typhimurium* to *S. paratyphi B*, or vice versa, by genetic transduction, using bacteriophage as a vector of genetic material. Since we can thus, in a sense, hybridize the species, it is at least theoretically possible that we might

discover the bacterial character concerned in the difference in mouse pathogenicity by examining the pathogenicity of hybrid strains showing various combinations of parental characters.

The problem of host-specificity, which is in a sense the problem of the less obvious bacterial characters required for virulence, is accessible to experiment in such cases, when a susceptible experimental animal is available. A successful analysis might then assist us to discover the basis of the ability of, for instance, the gonococcus to infect man, but none of the usual laboratory animals.

BACTERIAL GENETICS AND ACTIVE IMMUNIZATION

In many bacterial infections the disease process is terminated, and the host is rendered partly or totally immune to further attack, by a specific immunological response of the host to one or more antigens of the bacterium, that is, by the production of specific antibodies; and we may wish to protect susceptible persons by active immunization. In a few cases, in particular diphtheria and tetanus, we can immunize with a well identified bacterial product, but in most cases no such single "protective antigen" is available, and we must rely on the administration of whole bacterial cells (or cultures), either killed, or living but avirulent. The importance of a genetic approach to the selection of strains of good immunizing potency for use as dead vaccine is evident, and I shall not discuss it here. In some infections, however, only the administration of live vaccines has been found effective in immunization. The problem then is to find a bacterial strain which can be relied upon to give an adequate immunological stimulus to the host, but never itself to cause a serious pathological process. We require a nonvirulent strain which can be guaranteed *never* to revert to virulence; if even a single virulent bacterium arising by reversion were present in the vaccine inoculum it would presumably multiply in the inoculated host and cause a progressive infection. Bacterial strains which can be used as live vaccines have been obtained, in the main, empirically. The BCG strain of tubercle bacillus and certain strains of the plague bacillus have been extensively used for human immunization, despite lack of knowledge of what bacterial characters are responsible for their lack of virulence. It is interesting to contrast this with the position in respect to immunization of animals against anthrax. It is known that all virulent strains of *Bacillus anthracis* produce capsules *in vivo*, and Sterne (18) showed that all such strains also produced capsules *in vitro*, provided they were grown in the presence of serum and of a raised concentration of carbon dioxide. The capsule of the anthrax bacillus, which is composed of a polymer of D-glutamic acid, protects the bacillus against phagocytosis, and this presumably is why its presence is essential for virulence. Sterne (18) showed that it was easy to isolate nonencapsulated variants, or mutants, from virulent strains, and that live spore suspensions of such variants were satisfactory immunizing agents. Live vaccines of this type have been very widely used for a number of years; the vaccine strain has never been known to revert to virulence. We have here an example of the successful use of an avirulent mutant as a live vaccine, in which we know the physiological basis for the absence of virulence. We do not, however, know why these nonencapsulated mutants of *B. anthracis* never regain their encapsulation. In other genera, e.g., in the pneumococcus, some nonencapsulated variants have a low, but detectable, rate of back mutation to encapsulation, so that injection of very large numbers of the nonencapsulated strain into mice sometimes causes death, an encapsulated culture being recovered at post mortem examination. Very little systematic genetical work has been done on the *Bacillus* group, and no recombination method is available for testing whether, for instance, the stable nonencapsulated mutants result from loss of a whole segment of chromosome, as appears to be the case in some nonreverting biochemical mutants in Salmonella.

The successful use as a live anthrax vaccine of a nonvirulent strain which owes its lack of virulence to a mutation which does not impair its immunizing efficacy suggests that it might be possible to prepare equally safe live vaccines in other genera. In Salmonella, for instance, there are available the types of biochemical mutant mentioned above, which are nonvirulent because of their nutritional requirements and yet retain the normal antigenic structure. In *S. typhimurium* the use of some such mutants as vaccines has been shown to confer some protection on mice. It might be possible to prepare similar mutants in, for instance, the tubercle bacillus. However, the great difficulty in getting properly controlled tests on the efficacy in man of even existing vaccines, e.g., typhoid vaccine and BCG, suggests that even if such 'made to measure' strains were available it would be difficult to determine their value for human immunization.

BACTERIAL GENETICS AND CHEMOTHERAPY OF BACTERIAL INFECTIONS

It frequently happens that bacterial infections cannot be successfully treated with certain antibiotics or other chemotherapeutic agents, because the infecting bacteria are, or during treatment become, resistant to the agent, even though they belong to a species regarded as universally sensitive to the agent concerned when it was first introduced. This replacement of populations of sensitive bacteria by populations of resistant ones provides a beautiful, but inconvenient, illustration of the phenomenon of evolution through natural selection, and because of it the genetic analysis of drug-resistance in bacteria is of great practical importance. It has also been of theoretical importance in the development of bacterial genetics, because of the need to resolve the conflict as to the mechanism by which a bacterial strain "becomes resistant" when cultivated in the presence of an anti-bacterial agent. In earlier years it was generally believed that the heritable increase in resistance found in a bacterial culture which has been "trained" by growth in the presence of an anti-bacterial drug resulted from a specific change (resistance) in the hereditary constitution of the individual bacteria directly caused by the action of the drug. The researches of the bacterial geneticists, culminating in the elegant method of indirect selection (Lederberg & Lederberg [14]) have, however, proved that heritable resistance of a culture to a drug can arise from the selection by the drug of resistant mutants, resulting from spontaneous mutations which occur in the presence or the absence of the drug. In my opinion spontaneous mutation and selection can account for all the observed phenomena of acquired heritable drug-resistance in bacteria; there is, I believe, no instance in which it has been conclusively proved that heritable resistance has been produced by a specific change in the hereditary character specifically induced by the drug concerned.

One may ask what bearing this theoretical conclusion has on the practical problems of drug-resistance. The nature of the problem varies from one system to another. Only in a few situations does it happen that an infecting population of sensitive bacteria are replaced during treatment by resistant bacteria which are descendants of the original sensitive ones, rather than the result of a secondary infection. However, this may occur in several different kinds of bacteria, when streptomycin is used. There is every reason to suppose that the mechanism then is simply the selection of streptomycin-resistant mutants, resulting from spontaneous mutations occurring during the multiplication of the sensitive bacteria in the patient. The clearest evidence is from the experiments of Garrod (8) in the early days of streptomycin therapy. He examined the urine of patients suffering from acute urinary infections caused by streptomycin-sensitive coliform organisms. Very large numbers of bacteria, e.g., 10^{10}, obtained by concentrating the bacteria from urine collected before treatment, were plated in streptomycin agar; a small number of colonies developed in some cases, indicating that streptomycin-resistant organisms, pre-

sumably mutants, were present in very small numbers even before treatment was begun. The probability of cure by streptomycin seemed (in the limited number of cases) to be inversely related to the frequency of these resistant cells. There are other antibiotics in which treatment may fail because of the appearance of resistant mutants during treatment, for instance erythromycin. However, in the case of penicillin and various other agents there is, in most infections, no such risk, or only a negligible risk. The problem of drug resistance, for instance of penicillin resistance in staphylococci, is then that of patients who are primarily infected, or superinfected during treatment, by resistant strains which have become common in hospitals and elsewhere since the agents concerned (penicillin, etc.) came into general use. In the case of yet other pairs of drug and bacterium, there is, surprisingly, no problem of drug resistance; for instance, no human infections by penicillin-resistant strains of group A streptococci or *Treponema pallidum* have been reported, as far as I know.

The study of the genetics of drug resistance explains some of these varying situations. The emergence of streptomycin-resistant descendants in the course of streptomycin therapy can be explained by the laboratory observation that bacteria of many different genera undergo mutation to high resistance to streptomycin, in a single step, at rates of about 10^{-10} per bacterium per generation, these mutants commonly resembling their parent in most other characters including virulence. In the case of penicillin, on the other hand, laboratory studies show that only a small increase in resistance can be achieved by a single mutation and that mutations which confer increased resistance often also cause decreased virulence. We can thus explain the difference between the streptomycin and the penicillin situations by showing that a particular kind of mutation occurs at a measurable rate in the one case and not in the other. It remains to be discovered why streptomycin-sensitivity can be lost by a single mutative step, but not penicillin-sensitivity; this is a problem in physiological genetics which is not likely to be solved until we know a lot more about the mode of action of these antibiotics.

We are less well informed as to why, for instance, penicillin-resistant coagulase-positive staphylococci have become prevalent, whereas penicillin-resistant group A streptococci have not—despite the fact that mouse-virulent penicillin-resistant variants have been obtained from group A streptococci in the laboratory (Rosendal [16]), whereas penicillin-resistant staphylococci of the kind which cause trouble in practice (i.e., strong penicillinase-producers) have never been conclusively shown to arise from sensitive strains, though the reverse change, mutative loss of ability to produce penicillinase, and so of penicillin resistance, occurs frequently (Barber [3]). A possible explanation is that a small minority of penicillin-resistant coagulase-positive strains of staphylococcus existed before penicillin came into use and have since become prevalent as sensitive strains are eliminated; but that no such strains of group A streptococci existed and that to obtain penicillin resistance in this organism, without loss of virulence, requires a large number of successive mutations, first-step mutants not being sufficiently penicillin-resistant (or virulent) to be selected during the penicillin treatment of infections or in penicillin-contaminated environments.

An understanding of the genetic mechanisms of drug-resistance can be of practical value in this field. When the use of an agent may fail because of the appearance of resistant mutants during treatment, e.g., in streptomycin therapy, the simultaneous use of a second agent, resistance to which can only be acquired by a separate mutation, is theoretically valid and seems to have been successful in practice. If it can be shown that the replacement of sensitive organisms by resistant ones during treatment results, in a particular situation, not from selection of resistant mutants but from secondary infections by resistant strains, then the clinician

knows that precautions against cross-infection must be improved. The increasing prevalence of antibiotic-resistant strains in hospitals and the community at large is a problem in population genetics which requires much further study. On theoretical grounds it seems probable that if the use of, e.g., penicillin, was abandoned, not only in medicine but also in animal husbandry, etc., penicillin-sensitive strains of staphylococcus would gradually become more prevalent again, that is there would be a trend back toward the population equilibrium of sensitive and resistant strains which presumably existed before the introduction of the agent. However, it is very unlikely that such a step could be taken, even if it were considered desirable; it would seem advisable to discover more about the way in which resistant strains are selected. Gould (10) has recently shown that the air in casualty departments and the noses of hospital staff are contaminated with significant amounts of penicillin, and it seems possible that such contamination, which is in theory avoidable, and the selective conditions for resistant staphylococci which it creates in the anterior noses of hospital staff, is a much more important cause of the prevalence of penicillin-resistant strains than is the selective medium provided by patients under treatment.

USE OF GENETICALLY "LABELED" BACTERIAL STRAINS IN THE INVESTIGATION OF INFECTIONS

Minor bacterial characters, genetically stable and in themselves without effect on pathogenicity, may serve to split up a bacterial species into a series of sub-units, as for instance the phage-types of *Salmonella typhi*. This is often of great practical value. Minor antigenic characters, pattern of phage sensitivity ("phage-type"), and capacity to ferment particular sugars have been the most commonly used characters. It now seems that a number of other characters which have been of interest to the bacterial geneticist may be of value to the epidemiologist. For instance, strains of *Escherichia coli* may be differentiated by their sensitivity (inhibition of growth) to various amino acids (Rowley [17]), and strains of enteropathogenic *E. coli* and of *Shigella sonnei* by their production of different colicines (i.e., antibiotics active on certain strains of coliform bacteria); the latter character appears to be of value in epidemiological analysis, (Frédéricq, Betz-Bareau, and Nicolle, [7], Abbott and Shannon [1]). The gonococcus and the tubercle bacillus have thus far received little attention from bacterial geneticists, presumably because they are technically inconvenient material; but a search for suitable characters might make it possible to assign strains of gonococcus and tubercle bacillus (human variety) to types, which might be of use in attempts to control the diseases concerned.

One may also use these banal characters for the preparation, in the laboratory, of genetically labeled sub-strains of a particular strain, for use in experimental investigations of infection; recognizable sub-strains may be obtained by selection of mutants or by some method of genetic recombination. If several such sub-lines of a strain do not differ in pathogenicity or in rate of multiplication *in vivo* (if, in fact, the genetic "label" is no more than a label), they can be used to test, in a particular system, certain general hypotheses as to the mechanism of the production of infection. In many systems a very large number of live bacteria must be administered to elicit an infective response (fatal infection, local lesion, etc.) in 50 per cent of the hosts tested (i.e., the ED_{50} is large); this may be true even when the bacterial strain has been repeatedly passaged to select a variant of the maximum possible virulence. For instance, when mice are given inoculations intraperitoneally of *Salmonella paratyphi B*, the ED_{50} for a fatal infection is about 7,000,000 bacteria. There are two general hypotheses about this sort of situation, which I shall state as applied to the example selected. According to one, there is for each mouse a *Minimum Lethal Dose* (M.L.D.), i.e., a number of injected

bacteria which is just sufficient to swamp its defense mechanisms, so that if any larger number is inoculated all or many of them will multiply and cause a fatal infection; in the example stated the modal value of this M.L.D. is 7,000,000. The alternative hypothesis, of *Independent Action* (I.A.), states that every inoculated bacterium has a small chance of multiplying in the mouse so as to cause a fatal infection, and that for each individual bacterium inoculated this probability is unaffected by the number of other bacteria inoculated: if each inoculated bacterium has only a one in ten million chance of multiplying, it will be necessary to inoculate about seven million bacteria to achieve a one in two probability that one, two, or more of them will multiply and kill the mouse. Either of these hypotheses can account for the observed relation between dose and mortality. They differ in the way in which they account for fatal infections resulting from inocula just sufficient to kill, say one LD_{50} or less: according to the hypothesis of the M.L.D., mice dying as a result of inoculation of one LD_{50}, i.e., 7,000,000 bacteria, die because many of the inoculated bacteria multiply; but, according to the hypothesis of I.A., they die because a very small number, most probably one, of the inoculated millions chance to multiply. This antithesis permits an experimental test, by the use of an inoculum containing equal parts of several labeled sub-strains, say *a*, *b*, and *c*. If the hypothesis of the M.L.D. is applicable, mice which die from inoculation of one LD_{50} (comprising 2,700,000 each of *a*, *b*, and *c*) die as a result of multiplication of *many* of the inoculated bacteria, and the infecting population will therefore comprise all three sub-strains in about equal numbers; but, if the hypothesis of I.A. applies, most mice dying after inoculation of one LD_{50} (7,000,000 bacteria) die as a result of the multiplication of only one bacterium, and the infecting population will therefore consist of only *one* sub-strain, so that some mice will give, at post mortem, a pure culture of sub-strain *a*, others of sub-strain *b*, and so on. This mixed-inoculum experiment was, I believe, first used by Zelle, Lincoln, and Young (21), who infected guinea pigs with anthrax by inhalation of spores. The spore suspension contained equal parts of two variants differing in colonial morphology, but of equal virulence; many guinea pigs which died yielded at post mortem pure cultures of one variant or the other, as predicted by the hypothesis of independent action. Meynell and Stocker (15) applied the test to mice inoculated intraperitoneally with *Salmonella paratyphi B;* they used variants differing in their flagellar antigens, obtained by transduction. The results obtained were not clear-cut, but were interpreted as indicating the applicability of the hypothesis of independent action, with some modifications. Gorrill (9) has used this kind of test in an investigation of the effects of intravenous inoculation of staphylococci into mice; the mice survive the inoculation of large numbers of bacteria but develop renal abscesses, the total number of abscesses being very much fewer (about 100,000-fold) than the number of bacteria inoculated. When the inoculum contained a mixture of streptomycin-sensitive and streptomycin-resistant sub-strains, the large majority of the resulting abscesses gave pure cultures, of either sensitive or resistant bacteria, each mouse having abscesses of both types. This presumably indicates that the large majority of inoculated staphylococci are disposed of by the mouse but that a small minority produce abscesses in the kidney, each such abscess resulting from the multiplication of a single bacterium.

The use of genetically labeled strains may perhaps prove useful in the investigation of many other bacterial infections.

BACTERIAL GENETICS AND EPIDEMIOLOGY

The epidemiologist is concerned with the question of what natural mechanisms regulate the degree of virulence for the normal host of strains of pathogenic species and of the role, if any, of changes of virulence in the production of epidemics, etc. For most bacterial infections of man we have but little

information on this matter (see discussion by Wilson and Miles [20]) and cannot easily obtain more, through lack of a reliable experimental measure of "virulence for man." One can, however, make a theoretical analysis and correlate it with some relevant observations; such an analysis must be based on the results of experiments on the genetics of virulence measured in an experimental animal, even though these have of necessity involved highly "artificial" conditions, e.g., inoculation by intraperitoneal injection. Such studies have established that the virulence of a strain, as measured by a particular test, is subject to quantitative and qualitative variation; that serial passage of a strain from host to host commonly results in some increase in virulence for the host species concerned, it may be presumed because the procedure selects mutants of increased virulence; that strains of enhanced virulence thus obtained may be of unchanged, or even diminished, virulence when tested on a different host species, or by a different route. It is also known that strains maintained by serial culture *in vitro* often, but not always, decrease in virulence, the diminished virulence frequently reflecting a genetic heterogeneity, with an increasing proportion of the culture consisting of nonvirulent, or less virulent, variants. The population genetics of these changes have not been fully investigated; spontaneous mutation suffices to explain the origin of the less virulent forms, but it is probable that their rapid increase within a strain results from their better adaptation to the *in vitro* environment, for instance, from more rapid growth than the parental strain. When a strain is maintained in a liquid medium the genetically different components, e.g., smooth and rough, may interact in complex ways, the metabolic products of one component affecting the growth or survival of another, as shown by the extensive researches of Braun and his collaborators in *Brucella* (see Braun [4]). In a few instances, continuous *in vitro* passage of a strain of low virulence results in an increase in virulence (Felton and Dougherty [6]).

When considering the mechanisms regulating the virulence of strains in nature, rather than in the laboratory, one must first consider the ecological situation. Some human infections, e.g., gas gangrene, are purly "accidental" from the point of view of the bacterial strain, since the infection does not contribute to the dispersal of the strain; in such systems virulence for man is an accidental concomitant of some feature, e.g., the production of an enzyme, which may have survival value in the normal ecological niche, e.g., the soil. I shall discuss the opposite case, i.e., bacteria which multiply only as parasites of man, e.g., the typhoid bacillus. It seems clear that in the long run natural selection will here favor a parasite which seldom or never kills its host, and that there will be an evolutionary trend toward "low virulence" in this sense. Acute cases of typhoid fever mostly result from the dissemination of *Salmonella typhi* by so-called carriers, who in fact suffer from a chronic infection, commonly of the gall bladder, which causes only mild symptoms; it appears, then, that the typhoid bacillus has evolved to a state in which it is well adapted to prolonged coexistence with a human host, and it is perhaps surprising that when it infects a new host it should cause such a severe, frequently fatal, disease as typhoid fever. I suggest that the explanation may be that the typhoid bacillus, though well adapted to cause a chronic, nonlethal infection of, for instance, a previously damaged gall bladder, is only enabled to reach this site by its capacity to cause a generalized infection when it infects a fresh host; natural selection will then insure that the species retains sufficient virulence to do this, and the death of a proportion of newly invaded hosts may be, for the species, an unfortunate but unavoidable consequence of this necessary degree of virulence.

Some typhoid "carriers" excrete mainly rough typhoid bacilli, which are avirulent and therefore unable to infect a new host. This illustrates the point that mutations to nonvirulence occur in nature, as well as in

the laboratory. *Bacillus anthracis* is probably in nature an obligate pathogen of animal hosts; yet it too produces completely nonvirulent mutants in nature, as well as in the laboratory, for Chu (5) was able to isolate nonencapsulated, and therefore avirulent, strains of *B. anthracis* from consignments of goat skin which also yielded virulent strains, and also from soil experimentally contaminated with blood from animals dying from anthrax.

In addition to mutants of diminished virulence, which will be eliminated in due course because of their inability to infect new hosts, other classes of mutants will arise in infecting strains. When a bacterial strain is kept in continuous growth in the laboratory, the proportion of mutants which grow neither faster nor slower than the parent strain gradually rises; this presumably happens also in strains which are multiplying *in vivo*. However, there are theoretical reasons (see above, hypothesis of independent action) for supposing that in nature the infection of a new host usually results from the multiplication in it of only one, or a small number, of the bacteria shed by the previous host (in the case of typhoid fever there is indeed epidemiological evidence—Kehr and Butterfield [13]—that many infections result from the ingestion of only a single typhoid bacterium); in consequence, each passage from host to host will generally entail the elimination of all the mutants which had accumulated in the strain in its previous host, in the same way that they are eliminated when a strain is "purified" in the laboratory by picking a single colony. There are observations which suggest that this occurs even in the case of highly mutable characters such as H antigen phase change in *Salmonella typhimurium* (see discussion in Stocker [19]).

The specific antibody produced by the host contributes to the elimination of the bacteria in "self-limited" infections; mutants lacking the original surface antigen, but of undiminished virulence, would be unaffected by this antibody, and so presumably would be at a relative advantage. It appears that the relapses in experimental relapsing fever result from the capacity of the Treponema strains concerned to produce a series of serologically distinct variants which are unaffected by protective antibodies directed against the original strain. It is perhaps surprising that the same phenomenon is not encountered in other genera, for instance the Salmonella.

One may ask what part, if any, the various mechanisms of genetic recombination in bacteria play in nature, and in particular in the evolution and epidemiological behavior of bacterial pathogens. There is good evidence that pathogenic strains may in nature become, or cease to be, lysogenic for particular phages, and since in some cases changes in virulence result, this process evidently has some importance. However, there is, in my opinion, at present no evidence that the other known mechanisms (mating, phage-mediated transduction, transformation by DNA, transfer of F factor and of colicinogenicity) have a role of any importance in epidemiology, since spontaneous mutation and selection can account for the observed instances of changes of hereditary character; their role in the evolution of bacteria is equally problematical. Their chief interest in the study of infectious disease is as tools for the investigation both of infectious disease and of the underlying problems of bacterial variation.

REFERENCES

1. ABBOTT, J. D., and SHANNON, R. A Method for Typing *Shigella sonnei*, Using Colicine Production as a Marker. J. Clin. Path., 11:71–77, 1958.
2. BACON, G. A.; BURROWS, T. W.; and YATES, M. The Effects of Biochemical Mutation on the Virulence of *Bacterium typhosum:* the Loss of Virulence of Certain Mutants. Brit. J. Exper. Path., 32:85–96, 1951.
3. BARBER, M. Drug Resistance of Staphylococci with Special Reference to Penicillinase Production. *In:* Drug Resistance in Micro-organisms: Mechanisms of Development, pp. 262–79. London: Churchill, 1957.

4. BRAUN, W. Bacterial Genetics. Philadelphia: Saunders, 1953.
5. CHU, H. P. Variation of *Bacillus anthracis* with Special Reference to the Non-capsulated Avirulent Variant. J. Hyg., Camb., **50**:433–44, 1952.
6. FELTON, L. D., and DOUGHERTY, K. M. Studies on Virulence II. The Increase in Virulence *in Vitro* of a Strain of Pneumococcus. J. Exper. Med., **39**:137–54, 1924.
7. FRÉDÉRICQ, P.; BETZ-BAREAU, M.; and NICOLLE, P. Typage des souches d'*Escherichia coli* de gastro-entérite infantile par recherches de leurs propriétés colicinogènes. C. R. Soc. Biol., **150**:2039–41, 1956.
8. GARROD, L. P. The Reactions of Bacteria to Chemotherapeutic Agents. Brit. M. J., **1**:205–10, 1951.
9. GORRILL, R. H. The Establishment of Staphylococcal Abscesses in the Mouse Kidney. Brit. J. Exper. Path., **39**:203–12, 1958.
10. GOULD, J. Environmental Penicillin and Penicillin-resistant *Staphylococcus aureus*. Lancet, **1**:489–93, 1958.
11. GRIFFITH, F. The Significance of Pneumococcal Types. J. Hyg., Camb., **27**:113–59, 1928.
12. ———. Serological Races of Pneumococci. *In:* A System of Bacteriology in Relation to Medicine, pp. 201–25. London: Medical Research Council, 1929.
13. KEHR, R. W., and BUTTERFIELD, C. T. Notes on the Relation between Coliforms and Enteric Pathogens. Pub. Health Rep., **58**:589–607, 1943.
14. LEDERBERG, J., and LEDERBERG, E. M. Replica Plating and Indirect Selection of Bacterial Mutants. J. Bact., **63**:399–406, 1952.
15. MEYNELL, G. G., and STOCKER, B. A. D. Some Hypotheses on the Aetiology of Fatal Infections in Partially Resistant Hosts and Their Application to Mice Challenged with *Salmonella paratyphi B* or *Salmonella typhimurium* by Intraperitoneal Injection. J. Gen. Microbiol., **16**:38–58, 1957.
16. ROSENDAL, K. Investigation of Penicillin-resistant Streptococci Belonging to Group A. Acta Path. microbiol. Scandinav., **42**:181–88, 1958.
17. ROWLEY, D. Interrelationships between Amino-acids in the Growth of Coliform Organisms. J. Gen. Microbiol., **9**:37–43, 1953.
18. STERNE, M. Variation in *Bacillus anthracis*. Onderspoort J. Vet. Sc., **8**:274–349, 1937.
19. STOCKER, B. A. D. Measurement of Rate of Mutation of Flagellar Antigenic Phase in *Salmonella typhimurium*. J. Hyg., Camb., **47**:398–413, 1949.
20. WILSON, G. S., and MILES, A. A. Herd Infection and Herd Immunity. *In:* Topley and Wilson's Principles of Bacteriology and Immunity, 4th ed., pp. 1437–60. London: Edward Arnold, 1955.
21. ZELLE, M. R.; LINCOLN, R. E.; and YOUNG, G. A. Respiratory Pathogenicity of *Bacillus anthracis* Spores. II. Genetic Variation in Respiratory Pathogenicity and Invasiveness of Colonial Variants of *B. anthracis*. J. Infect. Dis., **79**:247–53, 1946.

Genetic Aspects of Tissue Transplantation and Tolerance

RAY D. OWEN*
Biology Division, Oak Ridge National Laboratory,† Oak Ridge, Tenn.

Nowhere is the diversity among the individuals of a species more evident than in the field of tissue transplantation. Surgeons today consider it axiomatic that a skin graft from one person will be rejected by any other person, unless the donor and recipient are identical twins. In fact, acceptance of skin grafts exchanged between two persons now has medicolegal status as evidence that the two persons in question are identical twins. The fact that identical twins constitute the universally recognized exception to the incompatibility of tissue transplants between individuals reflects the recognition that this kind of variation is inherited; it is their genetic identity that provides these twins with a unique basis for tissue compatibility (cf. Medawar [51]).

The mechanism of tissue rejection is generally accepted as immunologic. An individual who rejects a tissue transplant is so sensitized by this experience that he rejects a second transplant from the same donor more rapidly than he did the first. This "immunity" can be passively transferred from one experimental animal to another by injecting spleen or lymph node cells from the immune animal into a normal recipient of the same inbred line (52). With the establishment of these immune cells, the injected animal acquires the ability to give the immune, "second-set" response to transplants of the sort against which the cells were sensitized.

We have, therefore, a common appreciation of individuality in tissue transplantation, individuality based on such great diversity of genetic constitution that two individuals, unless they are identical twins or members of the same inbred line, are rarely alike. The genetic variation is expressed as antigenic differences among individuals. Donor tissue possessing one or more antigens different from those of its host calls forth an immune response in the host and sets in train processes leading to the destruction of the grafted tissue. The immunologic part of this system is a matter of lively interest, but our attention here must be directed toward the nature and genetic control of materials responsible for initiating the immune response, and not with the response itself.

The speakers at this symposium were urged to generalize in the treatment of their subjects. I am in sympathy with that estimate of what is proper here, but hope that the many scholars who have contributed in important ways to this field, and who are currently making contributions, will understand and forgive the uneven recognition that is inherent in a paper of this kind. The literature citations include several reviews and research reports that do a better job of giving recognition where it is due.

GENERAL CONSIDERATIONS

As one might expect, recognition of individual diversity in tissue transplantation

* On leave, 1957–58, from the Division of Biology, California Institute of Technology, Pasadena, Calif.

† Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

antedates any attempt to analyze or explain this diversity in modern genetic terms. Among the earlier clear encounters with the phenomenon were experiences with incompatibility in parabiosis, in the mid-19th century (see Finerty [21]). As early as 1905 Ehrlich recognized immunization by a tumor transplant in experimental animals, and in 1912 Schoene (64) reported that rabbits given injections of tissue preparations from other rabbits destroyed subsequent skin grafts from these donors more rapidly, whereas skin "autotransplanted" from one site to another on the same animal was not affected. Tyzzer, in 1909 (76) concluded that susceptibility to a particular carcinoma was inherited, but this inheritance did not seem to obey Mendelian laws. Almost all hybrids between a resistant and a susceptible group of mice were susceptible to the test carcinoma, as though susceptibility were dominant in a Mendelian sense. But in the next two generations, no susceptible mice were found. This disappearance of apparent dominance was difficult to comprehend in Mendelian terms.

In 1914, Dr. C. C. Little (38), then just completing his graduate study at the Bussey Institution, published a note in *Science* showing that the kinds of ratios encountered by Tyzzer and by Little in Tyzzer's laboratory were to be expected if susceptibility and resistance resulted from the interaction of numerous different dominant factors. In 1916, Little and Tyzzer (44) reported on a series of 629 mice given inoculations of a particular tumor and developed a concept for the genetic basis of tissue incompatibility in modern terms. Dr. Little has reviewed the subject to 1941 (40), and Snell (69) has given a brief account of the development of the field of tumor transplantation at the Jackson Memorial Laboratory, from which many of the most important contributions to this field have come.

The transplantation of normal tissues was subjected to the same kind of analysis by Little and Johnson in 1922 (42) and extended by other workers, particularly by Loeb and Wright (45). Rogers (63) presents a brief review of parallel and concurrent developments, particularly in Germany. Thirty years ago, therefore, at least the broader outline of current genetic concepts of tissue incompatibility had been defined.

RESISTANCE TO TUMOR TRANSPLANTS

The relative ease of tumor transplantation and the availability of a conspicuous and objective assay for the success or failure of the transplant have made tumor transplantation a most productive technique for the evaluation of the genetic control of tissue incompatibility. In general, a host can cause a strongly incompatible tumor to regress, whereas a relatively compatible tumor grows progressively and causes the death of its host. The availability of a large selection of reasonably stable mouse tumors and the increasing availability of highly inbred lines of mice, with virtually identical genetic constitutions within each line and marked genetic differences between lines, have provided other necessary components of a productive test system. On the other hand, the occurrence of escapes of either host or tumor, with different frequencies depending mainly on the particular host-tumor combinations under study, often complicates this kind of analysis.

Table 1 illustrates the classic experience in this field. Little and Tyzzer found that a mammary adenocarcinoma of a strain of Japanese waltzing mice grew progressively when transplanted to other mice of the same strain. Common mice were all resistant to implants of this tumor. Hybrids between susceptible and resistant mice were virtually all susceptible, suggesting a "dominance of susceptibility" to the tumor. Of the F_2 hybrids, however, only three were susceptible out of a test population of 183. All backcrosses to the susceptible parent were susceptible, whereas backcrosses to the resistant parent were all resistant.

The genetic basis for this kind of result is conceived as follows: Suppose that the tumor has a gene, *A*, controlling the presence of an antigenic specificity that will induce an immune response leading to regression of the

tumor in any host lacking this gene (*aa*). If the Japanese waltzing strain is *AA* and common mice are *aa*, their hybrids will be *Aa*, and the tumor will grow in them. The F_2 hybrids would segregate as three "susceptible" (having the *A* gene either homozygous or heterozygous) and one resistant (*aa*). Obviously, the assumption that a single gene difference between the parents is responsible for this tumor-host relationship does not explain the low frequency of susceptible mice in F_2. Suppose, however, that *two* independent dominants are present in the tumor, the absence of either of which in the host would make him resistant. The hybrids, *AaBb*, would again be susceptible, but in F_2 only nine of sixteen would be expected to have both *A* and *B* and therefore to be susceptible to this tumor. The "percentage susceptible" in F_2 would have dropped 56.25. Again, the assumption of two factors is inadequate to explain the experimental data, but a start has been made in the right direction.

TABLE 1

GROWTH OF TUMOR JWA IN JAPANESE WALTZING MICE, IN COMMON MICE, AND IN VARIOUS HYBRIDS BETWEEN THESE TWO*

Stock	+	−	Per Cent +
Japanese waltzing mice	38	0	100.0
Common mice	0	99	0.0
F_1 hybrids	61	1	98.4
F_2 hybrids	3	180	1.6
F_1 hybrids×Japanese waltzing mice	63	0	100.0
F_1 hybrids×common	0	78	0.0

* From Little, 1941. *In:* G. D. SNELL (ed.), "Biology of the Laboratory Mouse," p. 280.

TABLE 2

THE RELATION BETWEEN THE PERCENTAGE OF MICE SUSCEPTIBLE TO A TRANSPLANTED TUMOR AND THE NUMBER OF GENES RESPONSIBLE FOR THE SUSCEPTIBILITY

Pairs of genes the simultaneous presence of which is needed	Per cent susceptible in F_1	Per cent susceptible in F_2	Per cent susceptible in backcross of F_1 × susceptible parent	Per cent susceptible in backcross of F_1 × nonsusceptible parent
1	100	75.0	100	50.0
2	100	56.2	100	25.0
3	100	42.2	100	12.5
4	100	31.6	100	6.2
5	100	23.7	100	3.1
6	100	17.8	100	1.6
7	100	13.3	100	0.8
8	100	10.0	100	0.4
9	100	7.5	100	0.2
10	100	5.6	100	0.1
11	100	4.2	100	0.05
12	100	3.1	100	0.02
13	100	2.3	100	0.01
14	100	1.7	100	0.005
15	100	1.3	100	0.002
16	100	1.0	100	0.001

* From Little, 1941. *In:* G. D. SNELL (ed.), "Biology of the Laboratory Mouse," p. 283.

Table 2 shows that the expected percentage susceptible in F_2 falls in a series $(3/4)^n$ as n, the number of independent pairs of genes postulated for this kind of

system, increases. The observation of 1.6 per cent susceptible in the F_2 of the classic case would be consistent with the assumption of about fourteen gene loci assorting independently and acting as assumed. Furthermore, in the backcross, the probability of susceptible individuals is lower for each number of postulated gene pairs, and falls in a series $(1/2)^n$. All backcrosses to the susceptible parent would be susceptible, but only five animals in 1000 in the backcross to the resistant parent would be expected to be susceptible, if fourteen gene pairs are segregating. "Resistant backcross" ratios have on the whole given results compatible with the conclusions from F_2 analysis. Both linkage and the presence of "weak" histocompatibility alleles conferring incomplete protection on the host toward a tumor result in underestimation of the true number of genes concerned.

The mice available for the early studies of compatibility in tumor transplantation were not good inbred lines, in the modern sense, and the groups of mice providing the basis for the segregating populations were very different from one another. In general, later estimates of the number of gene loci concerned with the transplantation of particular tumors have been lower than the fourteen or so suggested in the classic study just cited. Soon afterward (39) data on a sarcoma of the Japanese waltzing mice indicated that only four or five genes were needed. Later, Little and Strong (43) studied two transplanted adenocarcinomas of DBA mice, in hybrid generations after crosses with A mice, and concluded that one of these tumors required two and the other three genes. Simultaneous reactions of animals to the two tumors showed that the two genes important to the first tumor were also involved in the second; only one additional locus was required for the second. In 1926, Strong reported a "one-gene" ratio in another combination and later a "four-gene" ratio in which one of the genes appeared to be sex-linked. An approach had been developed, therefore, through which not only could the number of genes involved in tumor transplantation compatibility be estimated, but the contributions of particular genes could be identified.

During the late 1940's, Dr. George Snell and his colleagues developed efficient methods for the identification and manipulation of particular loci involved in histocompatibility. At least two components of this important methodological contribution should be singled out for mention here. One is the development of "isogenic resistant" lines that may be assumed to differ only at the locus under study; the second is the use of linked, visible markers to follow the segregation of the locus in question. The method applied to the development of isogenic-resistant (IR) lines of mice is as follows: Given an inbred strain of mice designated as strain A and a tumor originating in this strain, to which the strain is susceptible, mice of strain A are crossed to any mice found to be resistant to the tumor. An F_2 generation is produced, and the tumor is inoculated into mice of this generation. Susceptible mice will be killed by the growing tumor, and only resistant mice will survive. These are mated back to strain A, and the procedure repeated for twelve or fourteen generations. Repeated backcrossing accumulates almost all the genotype of strain A in the selected mice, but the selection for resistance to the strain A tumor forces retention of histocompatibility alleles conferring resistance to the tumor. Snell reported that, under the conditions of this kind of selection, and in the great majority of instances, the difference between the original inbred strain and its essentially coisogenic mate ends up to be at a particular locus on the ninth chromosome known as the H-2 locus. The frequency with which differences at this locus turn up in this system of selection has been taken to indicate that alleles of this particular gene must play a predominant role in resistance to tumor transplantation.

The second method to be mentioned is the use of visible markers linked with histocompatibility loci, facilitating their identification and manipulation. In the late

1930's, Gorer showed that the A strain of mice had an erythrocyte antigen also present in fixed tissues that appeared to have an important relation to the fate of tumor transplants. In 1948, Gorer, Lyman, and Snell (27) found that the gene controlling this antigen was linked to a dominant gene affecting the tail, the gene "fused." The histocompatibility-erythrocyte antigen gene was at the H-2 locus already referred to. Later, Snell and his co-workers found that two other histocompatibility loci, H-1 and H-3, were linked, respectively, with the gene for albinism, *c*, in linkage group 1, and with the agouti locus, *A*, in linkage group 5. At least eighteen alleles have been identified at the H-2 locus, and at least three at H-3. Snell developed pairs or sets of coisogenic-resistant lines, permitting the study of effects of differences at these loci, probably uncomplicated by significant differences in the residual genotypes.

I shall not elaborate here the experimental procedures through which linkage with visible markers has been applied to the identification of histocompatibility differences. It should be sufficient to indicate that generations in which both the visible markers and histocompatibility are segregating can be inoculated with test tumors and data collected on the association of the marker characteristics with susceptibility or resistance to the transplant. Evidence for a close association with one of the complex of dominant tail characteristics on the ninth chromosome, for example, constitutes evidence that the histocompatibility locus segregating is H-2. The occurrence of recombination between the marker gene and the histocompatibility locus, the existence of "normal overlaps" in regard to the marker phenotype, escapes of either tumor or host in the tumor susceptibility test, and ratios grossly aberrant for unknown reasons frequently complicate interpretations from data of this sort.

Snell *et al.* (71) have now described another method for typing inbred strains of mice for histocompatibility antigens. This method depends on a system in which a known test tumor differs from a known host only at the H-3 locus. This is a "weak" difference, and under particular circumstances in a high proportion of inoculated mice the tumor grows progressively and kills its host in spite of the histocompatibility barrier. If, however, the host is first immunized by injection of tissue antigenically related to the tumor, the host is rendered capable of rejecting the inoculated tumor in a very high proportion of tests. When an unknown strain is used as the source of the immunizing tissue, this immunization will confer protection on the host if the donor material contains antigenic components related to the test tumor. The application of this system, therefore, provides a relatively rapid and sensitive basis for screening unknowns at this locus.

The years of patient and perceptive work that have been invested in the analysis of tumor transplantation genetics in the mouse have given us definitive data on specific genetic control, by individual genes, of transplantation compatibility. Even here, the number of loci thus far identified is evidently much smaller than the total number contributing tc this kind of individual variation within the species, and the extent of diversity at particular loci has only been sampled. Tumor transplantation offers opportunities for the investigation of numerous immunogenetic problems of significance in cancer research. The capacity of many tumors, in contrast to normal tissues, to override "weak" histocompatibility barriers and the wide variations in the breadth of "host range" among tumors naturally raise questions about basic aspects of the etiology and pathogenesis of cancer. Many of the studies of tumor-host relationships have a strong genetic component, related for example to variations in the number and kinds of chromosomes present in tumors, the histocompatibility factors, and their ultimate expression in the antigenic constitutions and interrelationships of hosts and tumors. The field is a large and forbidding one, on the level of our present consideration, and I shall not attempt to explore it further.

Several excellent reviews have appeared (26, 30, 41, 67).

TRANSPLANTATION OF NORMAL TISSUES

Although surgical experiences with the transplantation of normal tissues go back nearly a century, and although experimental designs directed at analyzing the genetic control of histocompatibility in normal tissues began to be applied soon after the pioneering work on tumor transplantation, much of the information available about the genetics of normal tissue transplantation derives, in a sense, from preceding tumor transplantation studies. Several references to earlier work in the transplantation of normal tissues were given in the introduction to this paper. Brief mention of later approaches to the genetics of this system, undertaken by a number of workers, will illustrate the current status of the subject. The special system of transplantation of tissues into irradiated recipients will be considered in another section of this paper.

Medawar, in 1945 (48) reported that reciprocal skin grafts among 22 rabbits were all unsuccessful and concluded that a minimum of seven independently combined antigenic differences would be required to provide this diversity. In the absence of breeding tests, of course, the genetic basis of these differences could only be speculative. The assumption of a single allelic alternative at each postulated histocompatibility locus in a population, and the further assumption that one of these alleles governs the dominant production of an antigen while its alternative is inactive and recessive, would clearly be unrealistic in terms of current knowledge of gene loci controlling antigenic specificities.

A step in a more precise direction, genetically, is illustrated by Hildemann's analysis (29) of incompatibility in transplantation of goldfish scales. The system is a remarkably convenient one, because numerous scales may be plucked from their pockets in individual fish and transferred to empty scale pockets in others, and experimental designs can thus provide complete "checkerboard" cross-testing of numbers of individuals. By restricting his studies to F_1 and F_2 generations of particular pair matings, Hildemann was able to restrict the number of possible alleles at any locus to a maximum of four (assuming that each parent may be heterozygous at the locus, and that the alleles present may all be different, i.e., $A^1/A^2 \times A^3/A^4$). Among 506 transplants, involving complete reciprocal exchanges among 23 F_1 sibs, Hildemann observed no successful transplants, although autografts were in every case accepted. In the F_2, a test of similar magnitude revealed no compatible combinations. The degree of diversity, even within a sibship and even after a generation of full-sib mating, is obviously great.

As in tumor transplantation, however, the most powerful material for analysis of genetic diversity in normal tissue transplantation is provided by inbred lines of mice. Here, crosses can be made between members of different inbred lines, and the F_2 and backcross progeny can be classified by means of skin transplants from a parent line. In this design, there is a further restriction on the kind of genetic diversity that may be present at a locus. Since the parents are members of highly inbred lines, they are presumably each homozygous at all the relevant loci. Accordingly, crosses between inbred lines may result in the introduction of only two alleles at a locus.

Again, the assumption generally made is that a graft will fail if it contains a gene controlling the production of an antigen different from any in the host. In extending calculations of "antigen number" to estimates of "gene number," the further assumptions are often made that there is a one-to-one relation between allele and antigen and that the members of an allelic series are co-dominant, as they often appear to be in the control of blood group antigens. Aside from its basis in the more thorough studies of tumor transplantation, the latter assumption rests mainly on the general experience that transplants of normal tissue from either inbred parent line to F_1 hybrids are uniformly successful, a result compatible

with an assumption that the heterozygous F_1 produces, or at least fails to respond to, all the antigens of both parents. Furthermore, skin from an F_1 hybrid is regularly rejected by either inbred parent, suggesting that at least some of the alleles inherited from either parent find expression, in the heterozygous condition, in antigenic differences recognized as "foreign" by the other parent. Although considerable experience supports these generalizations, it was only relatively recently that Eichwald and Silmser (19) observed that parent-to-F_1 skin grafts very frequently fail. The basis for failure seems in this instance to reside in antigenic differences between males and females, even within the same inbred, and presumably homozygous, lines. This observation has been confirmed and extended (66); often, but not always, male-to-female skin transplants in mice are unsuccessful. There has been some tendency to assign this phenomenon to an antigenic effect of the Y chromosome, but in the absence of definitive genetic evidence it would seem as reasonable to classify it as comparable to other secondary sexual characteristics that need not relate at all to the Y chromosome. The occurrence of some successful transplants from males to females within lines showing some degree of incompatibility in this combination can hardly be taken as evidence of genetic heterogeneity within the inbred line, but seems rather to suggest that the sex-histocompatibility effect may in these instances be weak and may or may not prevent the persistence of the transplant. We can anticipate with interest the development of more information about this provocative system.

The study by Eichwald and Silmser just referred to also illustrates another point now coming to general acceptance in the field. The experiment was designed for estimating the number of genes that might be involved in the control of skin transplant compatibility between two inbred lines of mice. Relatively early readings of the skin grafts resulted in the assumption that five or more genes were involved (20). More extended observation of the mice, however, revealed many examples of unexpected and late failure. It is evident that estimates of gene numbers in such a system will depend on the length of the period over which the success of transplants is evaluated; many "weak" histocompatibility differences come to expression only slowly. An excellent study by Barnes and Krohn (3) provides extensive quantitative data on this point. Calculating the number of genes involved in histoincompatibility for skin transplants between A and CBA strains according to the standard assumptions, and using survival for 100 days as an arbitrary criterion for compatibility, these authors concluded that certainly not less than fifteen independently segregating genes were involved. Breakdown of "successful" grafts was, however, observed as late as 180 days after grafting. On this basis, the estimate of fifteen gene differences must be considered too low. The distribution of survival times of the grafts was at variance with an assumption that quick breakdown resulted from the cumulative effect of larger numbers of histocompatibility differences between graft and host, whereas slow breakdown depended on fewer differences. As in other cases in the transplantation field, and as in such other parts of the field of immunogenetics as blood group determination, it is clear that different loci and alleles have different values in terms of effective antigenicity, and there is no simple additive relation among these, either in the evocation of an immune response or in the susceptibility of cells to the response. Another interesting point in the study by Barnes and Krohn is that the delayed reactions, once they are in train, seem to progress with reasonably constant rapidity. Potentially incompatible grafts may remain in place and apparently healthy for extended periods of time, but once their breakdown is initiated it is soon completed. What the basis may be, in the interaction of host and graft, for this long toleration of a foreign tissue followed by a relatively explosive reaction to it, remains an important problem for further research.

Prehn and Main (61) have also analyzed

histocompatibility differences between inbred strains of mice. They report calculations that the BALB/cAn and DBA/2 strains differ by approximately thirteen loci, and postulate a cumulative effect of "weak" histocompatibility differences. These workers (60) earlier raised the interesting possibility that the histocompatibility alleles were less effective in single than in double dosage, based on longer survival in one parent of F_1 tissue than of tissue from the other parent. The F_1 is, of course, heterozygous at loci for differences at which the parents are homozygous. There now seems to be considerable basis for doubt (3, 9) that this "dosage phenomenon" is general in the field of tissue transplantation. A dosage effect is encountered very commonly in blood groups.

Again as in tumor transplantation, the identification and manipulation of particular histocompatibility loci have lagged behind more general considerations of genetic diversity. It is here that the dependence on prior and pioneering work with tumors is most evident; the detection of specific loci in normal tissue transplantation is, as far as I know, entirely restricted to observations that loci identifiable in tumor transplantation studies also affect compatibility of normal tissues. Among these, the H-2 locus already referred to is again one of overwhelming prominence.

Snell's "isogenic resistant" sets of mouse strains have provided the most precise basis for the identification of particular genes with histocompatibility in normal tissue transplantation. These lines, used as co-isogenic pairs in which the residual genotypes may be assumed to be virtually identical except for a locus selected by successive generations of tumor transplantation, also display incompatibilities in skin graft exchanges (16). It turns out that a difference at the H-2 locus alone may result in rejection of skin grafts about as rapidly as do the differences at numerous loci usually prevailing between ordinary inbred strains. Strain-pair differences at only the "weak" H-3 locus permitted considerably longer survival of exchanged skin grafts—average survivals, in fact, 2 or 3 times the 13 days maximum found for differences at the H-2 locus. In contrast to the observation by Barnes and Krohn previously cited, the course of the reaction was also slower and less severe, as well as delayed in its onset, in the "weaker" antigenic difference. A difference at the H-1 locus gave highly variable graft survival times, ranging from 10 days to as long as 112 days. Variations in technique may have been partly responsible for this wide range of survival times.

As Snell pointed out, these and similar findings suggest that significantly prolonged homograft survival may be attained "with only a partial matching of the genotypes of donor and host." Applying this consideration to clinical homografting in man, one may suggest that the genetic correlations between relatives may frequently result in compatible genotypes at major histocompatibility loci, and, although minor differences may still result in the ultimate rejection of the transplant, this rejection may be considerably delayed. Lindsley[1] calculated that, with the standard assumptions of relations between gene and antigen, the probability of compatibility at any locus is higher for full sibs than between parent and offspring. This probability of course drops off rapidly as lesser degrees of relationship are considered. Nevertheless, if Hildemann's extensive experiences with scale transplantation within sibships of goldfish, even after a generation of full sib mating, may be accepted as a model of the population situation for skin transplantation as well, the probability of a compatible transplant, or even of a degree of compatibility permitting significantly prolonged survival, between sibs is so small as to discourage random trials in a search for compatible donors in a clinically important situation. What is obviously needed is some practical test basis for "transplantation-typing" large numbers of individuals. Even given such a test, however, we may well wonder whether fully compatible combinations may not be

[1] D. L. Lindsley, personal communication, 1957.

so infrequent as to prove of little practical significance.

There is, as yet, no real "genetics" of tissue transplantation in man; genetic considerations have been largely restricted to the comparison of fraternal and identical twins and to calculations of possible frequencies of successful transplants (63). It is conceivable that current studies of human tissues in tissue cultures may eventually provide tools for a satisfactory genetic analysis (59).

A remarkable aspect of the immune phenomena associated with the "second-set response" in normal tissue transplantation is its apparent specificity for the particular combination of host and donor involved in the original sensitization. A general rule cannot be stated at this time, but there have been repeated indications that, although a host sensitized by the rejection of an incompatible skin transplant will give a second-set response to a repeated transplant from the same donor or strain, only a first-set response often occurs on implantation of tissue from a different donor or strain. Perhaps the most straightforward data of this sort are reported by Barnes and Krohn (3), who found that F_2 mice from crosses between A and CBA, after rejecting A skin, gave reactions of the first-set type to subsequent grafts of CBA skin. In this experimental design, such a result need not be surprising, if it is assumed that an F_2 mouse will react to the skin from one parent only on the basis of homozygosity at particular histocompatibility loci for the alleles contributed by the other parent; i.e., only if an allele present in the test graft is lacking in the test host. A later skin graft from the other parent to this mouse would require for its reaction a completely different set of loci, those at which the mouse was homozygous for the alleles from the *first* parent. A first-set response to the new antigens introduced by the graft from the second parent might therefore be expected. This is not to say, of course, that the antigens involved are necessarily in one-to-one relation to the alleles, but only that the genetic constitution of the host must place close restrictions on the kinds of graft specificities to which he will respond. It is more surprising to find that skin grafts exchanged between unrelated individuals may also fail to sensitize for a second-set response to different donors (49). Almost anywhere else in immunogenetics, one would expect to find shared or similar antigens distributed through the population in such overlapping ways as regularly to promote second-set responses under these conditions. If they do not, in fact, regularly occur, the situation may be somewhat reminiscent of that encountered in serotype serology of ciliates (47), where the absence of cross-reactivity among different cells and their corresponding antisera appears to result from a situation in which, in spite of a diversity of genetic potential, only a single serotype may be expressed by a cell at one time. Even here, however, antigenic specificities controlled by allelic genes appear to be subject to some degree of cross-reaction (4).

TRANSPLANTATION INTO IRRADIATED RECIPIENTS

One of the most active areas of tissue transplantation research involves the injection of potentially hemopoietic tissues into irradiated recipients. No detailed discussion of the rapidly growing literature in this area can be undertaken here; suffice it to say that 3 or 4 years ago several groups of workers independently and almost simultaneously discovered that bone marrow injected into an irradiated animal becomes established as a hemopoietic transplant (22, 37, 54, 78). X-rays, at relatively high dose levels, depress the host's ability to give an immune response, destroy his hemopoietic system, and create a "vacancy" in which the injected marrow settles and multiplies. If the donor marrow is "isologous" (that is, from the same inbred line), it persists without incident. If the donor tissue is either "homologous" (from a different inbred line or a genetically different individual of the same species) or "heterologous" (from another species, as, for example, rat into

mouse), the radiation has the effect of breaking down the initial histocompatibility barriers that would be set up by a nonirradiated host, and the transplant may become established and function. A high proportion of hosts so treated survive for considerable periods after radiation doses that that would otherwise quickly be lethal to them.

Particular interest here attaches to the delayed incompatibility that develops in animals treated with homologous or heterologous hemopoietic tissues (24). These delayed reactions frequently result in the death of the host, and their basis is being ardently debated at present. The application of certain "genetic" evidence in this debate provides our reason for discussing this subject here.

The controversy revolves around the question whether the delayed reactions result from a slowly recovering immune system in the radiologically damaged host, reacting with the foreign transplant, or whether the graft may be reacting against its histoincompatible host. The latter possibility has come to general attention largely through observations by Billingham *et al.* (7) of the effects of injecting adult spleen cells into newborn animals. Such injections result in the destruction of host lymphatic tissues, in a syndrome described as "runt disease," often leading to the death of the recipient and among survivors, in a persistent tolerance to skin grafts of donor type. The injection of homologous spleen cells into irradiated adults may also result in an extensive "graft-versus-host" reaction (65). Although it is a matter of common recognition, therefore, that spleen or lymph node cells are immunologically competent to produce such a reaction, the extension of this kind of explanation to the delayed effect of foreign bone marrow in irradiated hosts depends on the assumption that this tissue also normally contains a sufficient quantity and quality of immunologically competent cells to produce a fatal graft-versus-host reaction. The case for the negative in this debate has been based almost entirely on immunologic observations (46); for example, it has become evident that there is no arbitrary x-ray dose beyond which a surviving host fails to recover, in time, its capacity to give an immunologic response. In fact, in the bone marrow experiments, the period required for host recovery in this respect is closely correlated with the time at which a delayed incompatibility reaction appears. Furthermore, pre-immunization of the host with antigenic material related to that of the prospective graft results in regular early death of the irradiated and bone marrow-treated animals, an effect suggesting the induction of a secondary response in a host-versus-graft reaction. The delayed reaction does not correlate, in a positive way, with the dose of bone marrow in the irradiated recipient, as might be expected if the bone marrow were contributing antibody-producing cells. This is in contrast to the results when spleen is used instead of bone marrow, where one expects and observes that increasing the cell dose increases the speed and extent of the reaction. Furthermore, several lines of evidence[2] now suggest that cells derived from the established bone marrow are in an unfriendly environment; they display indications of the kinds of sensitization that might be expected if a recovering host mechanism were attacking them. Nevertheless, in many instances the pervasiveness of this kind of graft and its development in overwhelming quantity seem to render it capable of withstanding, or even of suppressing, significant host responses over long periods of time. Some general term like "immunologic paralysis" may be used to describe the host's condition over this interval. The occasional long-term survivors of even heterologous bone marrow transplantation are as yet unexplained; perhaps, in some circumstances, the host may become "tolerant" to the injected material. The host, however, does not regain normal immunologic reactivity unless he succeeds in sloughing the transplant altogether (25).

The argument for a graft-versus-host

[2] R. D. Owen, and T. Makinodan, unpublished data.

basis for the delayed death pattern (33, 75, 77) also has some immunologic components, though these, perhaps like the immunologic arguments just stated for the negative, have answers more or less persuasive, depending on who is evaluating them. The most compelling point, however, is a genetic one. It is asserted that, in bone marrow experiments involving reciprocal exchanges between inbred parents and their F_1 progeny, the delayed reaction occurs with greater regularity and severity when parent marrow is injected into irradiated F_1's than in the reverse. In terms of the usual experience in tissue transplantation, this should be considered a strong indication that graft-versus-host reactions are important in this system; the F_1 graft, if it has inherited all the parental antigens, should tolerate its parental host and should therefore fail to give a delayed reaction in the parent. Similarly, a parental graft should react to antigens inherited by the F_1 host from its other parent. The reverse situation would be expected if the delayed reaction were predominantly host-versus-graft.

This system is a relatively complex one, in which a number of variables in addition to histocompatibility differences between graft and host may be important. For example, the same absolute dosages of radiation produce different physiological levels of damage in different strains and hybrids; the LD_{50} and LD_{100} levels vary markedly from one strain or hybrid to another, and one can hardly be confident that effects on the immune mechanism are necessarily completely correlated with lethality effects of a given radiation dose. Furthermore, comparisons are to be made between different inbred lines, differing in their general vigor, and with F_1 hybrids that are typically much more vigorous than their inbred parents. This difference may well extend to the cellular population comprising the injected marrow. Although it seems probable that the primary reaction in the delayed death pattern has an immunologic basis, because the reaction does not occur when isologous marrow is used, the degree of immediacy between the initial reaction and eventual death of the host is not known. It is conceivable that under some circumstances the path from initial reaction to ultimate death may be indirect, involving, for example, the depletion by an antigen-antibody reaction of substances nonspecifically involved in protection against bacterial or virus infection, and resulting in eventual death by infectious disease. Furthermore, in this complex system, the use of inadequate marrow doses may have a profound influence on the ultimate effect; studies in which this variable is not controlled or uniform may give misleading results. Under the best of circumstances, the control of this variable is subject to reservation; counts may be made of nucleated cells in the inoculum, but, while we remain in ignorance of the particular cell types that may be causally related to either the protection or the delayed reaction, we cannot be sure that any two inocula are similar, especially when the inocula to be compared come from sources as different as inbred lines and hybrids. In addition, the assay here is a matter of how many hosts die at particular times, and the establishment of valid conclusions requires adequate statistical design, sampling, and test techniques. It is also important to consider the sexes of donor and recipient. Analogy with the skin transplantation studies previously cited in this connection makes it probable that F_1 females in some combinations would give a host-versus-graft reaction to parental male tissues, in apparent contradiction to the usual genetic "laws" of transplantation. Altogether, therefore, it is still possible to doubt that F_1 marrow in parents has a real and consistent genetic advantage over parental marrow in hybrids—the genetic basis of the graft-versus-host argument. We may, however, take the argument as granted for purposes of further discussion.

Our main concern, then, is with the conclusion that the genetic "laws" of transplantation point compellingly to a graft-versus-host mechanism for the delayed reaction. Here, it seems to me, some common caution is indicated by experiences elsewhere in the

field of immunogenetics. Even if it is assumed that the antigens responsible for the delayed death pattern in the bone marrow experiment are ordinary cellular antigens comparable in their inheritance to those more thoroughly investigated in the field of red cell immunogenetics, a general assumption that an F_1 hybrid between two homozygous parents will necessarily have all the antigens of both parents and no additional antigenic specificities has been contrary to facts established and generally recognized more than 20 years ago (32). More recent studies reveal the existence of genetically independent recessive suppressors of blood group antigens (36) and of interactions between alleles resulting in the appearance of "hybrid" specificities on cells (13, 15) and the interallelic suppressions at the Rh locus, discussed during this symposium by Ceppellini. These are among a variety of lines of evidence dictating caution in assuming too simple a relation between gene and antigenic specificity, particularly in a field in which so much remains to be learned as that of the immunogenetics of tissue transplantation.

When we turn from the cellular specificities themselves to other types of antigenic materials, such as the soluble antigens of human saliva, the control of specificities by single co-dominant alleles is a subject of equally long-standing and commonly recognized reservation. The recessive block to the secretion of blood group substances, genetically independent of the ABO blood group locus itself, has been known since 1930, and additional complex interactions among alleles and among independent loci affecting the character of these secretions have been discussed by Ceppellini at this symposium. Fox and his associates have reported indications of similarly complex effects in Drosophila homogenates, and I understand that Dr. Fox recently discussed this material with reference to its possible relation to tissue transplantation genetics (23). Even the antigens of cell surfaces may sometimes relate to these complex genetic situations, as witness, for example, the sequence of independent gene-controlled reactions affecting the antigenic character of red cells in sheep (62, 73). In the bone marrow experiments, in particular, it may be important to recognize that we are dealing with the establishment of a very diverse and pervasive tissue in irradiated recipients—cells whose progeny may have a great variety of functions and products. I would be surprised if it should prove that all the potential systemic antigens relate in a one-to-one way, each to a dominant allele, even if the establishment of the transplant itself should do so.

Although the available data on the transplantation of tumors and skin, as well as of several other normal tissues, seem to point to a usually direct relation between co-dominant alleles and the significant antigens, it seems to me still possible that different antigens, subject to a different kind of genetic control, may come into play under the conditions of the delayed reaction in bone marrow transplantation. Antigens peculiar to a parent may pile up behind recessive blocks in the inbred lines that must be homozygous for recessives at numerous loci, and heterozygosity in the F_1 hybrids may release these blocks, with the result that the F_1 may well *lack* specificities found in a homozygous parent, and may *have* specificities not found in either parent. Such a consideration is recognizably speculative at this time. We cannot point to known antigens displaying the patterns of inheritance I have just postulated and important in the delayed bone marrow reaction, though we know that such antigens exist in systems such as the human salivary secretions.

At present, our best judgment would probably favor the acceptance of the "genetic" evidence from reciprocal exchanges between parents and F_1 as supporting a graft-versus-host basis for the delayed death pattern. My point is that this evidence is not as yet completely compelling, and it should be considered as contributory rather than conclusive in its bearing on the mechanism of the delayed reaction. As often happens in scientific con-

troversy, it will not be surprising if proponents of both interpretations turn out to be right, and both graft-versus-host and host-versus-graft reactions prove important.

In concluding this section, brief further reference should be made to the best-known histocompatibility locus, H-2 in the mouse. As mentioned earlier, this locus appears to be involved in the control not only of histocompatibility reactions, but of antigenic characteristics of red cells as well. In the latter connection, the locus is subject to representation as a series of symbols, representing patterns of antigen-antibody reactions between the reagents at hand and the set of related cell types controlled by alleles at the locus. The interpretation of the relation of these symbols to the structure of the antigens themselves, and ultimately to the structure of the gene, is subject to the same kind of reservations as apply to similar complexes in cattle, fowl, and man. The locus appears to be peculiarly unstable (1, 31), and modified alleles derived from hybrids have been interpreted as cross-overs among adjacent loci (2). In the absence of genetic markers on each side of the locus establishing recombination as a fact, it would seem proper to reserve judgment on the origin of these mutation-like changes; modern genetics provides possibilities other than classic recombination for such effects.

As is true elsewhere in immunogenetics, a given allele at the H-2 locus may have an effect similar to that of heterozygosity for two others (70). Gorer and his colleagues have reported several remarkable phenomena associated with this locus; for example, antigenic specificities of the red cells of one animal but not expressed on the red cells of another may be present elsewhere in his body. What may be the genetic basis of this distributional variation within the organism and what other deviations may inhere in this locus from the classic simplicity of an earlier immunogenetics remain to be revealed. At present, it seems likely that information of the greatest interest for general genetics and immunology, as well as for the more circumscribed field of the immunogenetics of tissue transplantation, will come from work in progress on the H-2 and other histocompatibility loci of the mouse.

"IMMUNOLOGICAL TOLERANCE"

The final consideration will be of the genetic aspects of a system operationally the opposite of tissue transplant rejection, a system in which specific compatibility is conferred on a host, with respect to donor tissue toward which he would otherwise develop incompatibility. Several ways of suppressing immune reactions have been applied in the field of tissue transplantation—for example, "conditioning" hosts by cortisone treatment or irradiation, or both (74), and the preinjection of derivatives of prospective donor material, which may have effects either of immunization or of enhancement of the growth of tumor grafts depending on the dose of materials used in the preinjection (68). I have recently reviewed the general subject of suppression of immune responses (56) and shall not develop it in detail here. My concern will be restricted to the particular situation described as "immunological tolerance," the specific reduction in reactivity to homologous tissue transplants conferred on an animal by the introduction, during fetal or neonatal life, of homologous tissue cells. This area, a very active one at present, traces back to experiences of experimental embryologists, who have known for many years that grafts exchanged between embryos may persist for long periods of time. In chicks, even after hatching, homologous skin grafts may be accepted and tolerated indefinitely, as in the classic "Danforth preparation," studied rather extensively during the late 1920's (17).

It is not remarkable that the young animal fails to reject foreign tissue. The immunologic machinery, a relatively late developmental acquisition, fails to function effectively in the embryonic or neonatal period in many birds and animals. But why should foreign tissues established prior to the appearance of this machinery continue to be tolerated after the animal's capacity to

give an immune response should have matured? Furthermore, as the elegant experiments of Medawar and his associates have shown (6), the tolerant animal continues to accept new transplants of the donor sort. What is the basis of this persistent and specific modification of the histocompatibility "recognition-and-response" machinery, which up until this point in this paper has been considered the more or less direct result of the animal's genetic constitution? It is on the level of the genetic control of somatic development and differentiation that the subject of immunological tolerance offers its most exciting prospects and poses its most puzzling questions.

On the level of gene numbers and the activities of particular genes in tissue incompatibility differences among individuals, the simplest description of immunological tolerance would represent this phenomenon as a straightforward resultant of the histocompatibility characteristics of host and tolerated donor. The very young individual may accept the colonization of donor tissue cells, and be thereafter a chimera of host and donor cells; this happens naturally in a high proportion of fraternal twin cattle (55) and rarely in human fraternal twins (11, 18, 53). On this basis, the tolerant individual might be regarded as the sum of its host and graft components, a new entity capable of reacting normally to grafts distinct from it, but accepting without challenge grafts identical with either of the components of the entity. The reported specificity of immunological tolerance is compatible with such a conception (8). However, a statement of this sort is of course not an explanation of the phenomenon of immunological tolerance. It is rather a restatement of the problem in terms that describe the ultimate behavior of the individual but leave unanswered the questions of why and how foreign and potentially incompatible cells introduced into the young animal may prevent the development of, or suppress, the normal histoincompatibility reactions to these cells and to others like them later introduced.

"Runt disease" and the widespread and often almost total destruction of host lymphatic tissues often observed when adult homologous spleen cells are injected into young animals (5) would surely be expected to reduce the over-all immune responsiveness of the host in nonspecific ways. Studies of the general immune responsiveness of survivors of this treatment, comparable to the studies of the immune status of irradiated bone marrow-treated mice previously cited, might be of interest in this connection. Even in the survivors of runt disease, however, a residual specificity of reaction is evident. And tolerance is conferred by tissues other than adult spleen, which do not produce detectable destruction of host lymphatic tissue or cause runt disease. This effect is therefore not a necessary concomitant of immunological tolerance.

A recent study has shown that, in contrast to identical twins, fraternal twin cattle —presumably chimeras for each other's tissues and fully tolerant to skin grafts exchanged between them—give somewhat different responses to skin grafts from their dam. Since both members of such a twin pair are chimeras for the same two types of tissues, in which the roles of host and tolerated graft are simply reversed, it seems that some components of the tissue incompatibility reactions remain unique to the host, as a function of his particular genetic constitution, regardless of the presence of a tolerated transplant. Such a situation could be expected if a role is assigned to systemic antigens produced by tissues that are not mosaic, in the fraternal twins. A model is provided by antigen J (72), which is either present on or absent from all the red cells of a chimera as a function of the host genotype only. The response of twins differing in such an antigen to maternal tissues having the antigen would be expected to be different. The implication of antigens controlled in this fashion would, however, deviate from a principle currently prevalent in the field that the antigens involved in tissue incompatibility are, in both host and graft, direct functions of the nuclear constitutions

of the cells individually concerned and are alike for all cells of the same nuclear constitution. It should be evident that other bases for the asymmetrical response of fraternal twin cattle to maternal skin grafts can be conceived; perhaps another possible explanation would be the assumption that the immune systems of the twins are not effectively mosaic but are different in the two animals and react differently to maternal antigens not present in either twin.

A field of somewhat peripheral genetic interest, though of considerable general importance, upon which the concepts of immunological tolerance impinge, relates to the relationship between mammalian fetus and mother. Since the developing fetus is doubtless characterized by antigens distinct from any in the mother, the question of why the fetus does not evoke a "homograft reaction" has led to provocative discussions, particularly by Brambell (12) and by Medawar (50). In the direction fetus-into-mother, Rh sensitization, of course, provides evidence of breakdown of the usually effective mechanism through which maternal responses to fetal antigens, and subsequent immunologic effects on the embryo, must usually be prevented or suppressed. Levine's discovery (35), that ABO group compatibility of the mother toward fetal cells favors Rh sensitization, throws a new and different light on this interaction. It suggests that quick disposal of fetal antigens, rather than indifference to them, may sometimes be involved in the maternal "tolerance" toward the fetal "homograft." In cattle, Billingham and Lampkin (10) have recently reported frequent sensitization of dams to skin grafts from their progeny, presumably as a result of normal transplacental immunization of the dam. In the other direction, there has been some suggestive evidence of at least occasional tolerance conferred on embryos by the entry of maternal cells or antigens into them (8, 57). More recently, it has appeared that x-radiation during pregnancy might promote such a process; Hasek (28) refers to a finding that rats x-rayed *in utero* are rendered specifically tolerant to their mother's skin (not to their father's), and it has also been reported that the injection of isologous embryonic liver breis intraperitoneally into pregnant female mice, after relatively low doses of radiation, promotes the growth and survival of the young (58). The latter result was interpreted as evidence that a humoral principle from the injected liver traversed the placenta and promoted recovery from radiation effects, but the possibility of the establishment in the embryos of true chimerism as a result of the transplacental passage and establishment of intact cells from the injected liver brei has not been ruled out.

Another level of genetic interest relating to immunological tolerance is the possibility that "graft adaptation" in histocompatibility characteristics may sometimes be involved in this phenomenon. Although in particular systems changes in the graft may occur (14), adaptive changes in the transplanted tissue do not appear to be a necessary condition for tolerance. The changes that do occur, in some systems, and that may result from the selection of tolerated variants or, possibly, from a "type transformation" or "serotype transformation" of graft cells (34) are of the greatest genetic interest. Nevertheless, the fact that normal spleen cells injected into a tolerant animal cause him to reject the erstwhile tolerated transplant (8) indicates that the established graft continues to elaborate its own specific antigens, which are capable of evoking an immune response in normal cells, and that the graft remains vulnerable to the effects of such responses. Similarly, the maintenance of their original antigenic type by erythrocytes derived from exchanges of erythropoietic tissue in embryonic cattle, sheep, rats, chickens, and man and the absence of any evidence of cellular diversity that might result from the exchange of "transforming" materials at the subcellular level suggest strongly that the initial lesions of immunological tolerance are in the host's recognition and response machinery, and not in a change in the transplant.

The key problem, of course, remains that

of the nature of this lesion, and to this problem there is as yet no convincing answer. We do not know enough about the normal development and biologic nature of this machinery to do more than speculate about processes that might modify it in specific ways. A vague but exciting identification of both immunity and tolerance with genes and their actions in somatic development and differentiation is subtly current in this field. Perhaps it is well that our discussion should stop with an implication of that subtle excitement, a "feeling" that tissue transplantation and tolerance may throw new light on some of the most basic and challenging problems of biology.

REFERENCES

1. ALLEN, S. L. Linkage Relations of the Genes Histocompatibility-2 and Fused Tail, Brachyury and Kinky Tail in the Mouse as Determined by Tumor Transplantation. Genetics, **40**:627–50, 1955.
2. AMOS, D. B.; GORER, P. A.; and MIKULSKA, Z. B. An Analysis of an Antigenic System in the Mouse (the *H*-2 System). Proc. Roy. Soc., London, s.B, **144**:369–89, 1955.
3. BARNES, A. D., and KROHN, P. L. The Estimation of the Number of Histocompatibility Genes Controlling the Successful Transplantation of Normal Skin in Mice. Proc. Roy. Soc., London, s.B, **146**:505–26, 1957.
4. BEALE, G. H. The Genetics of *Paramecium aurelia*. Cambridge: Cambridge University Press, 1954.
5. BILLINGHAM, R. E., and BRENT, L. A Simple Method for Inducing Tolerance of Skin Homografts in Mice. Transplantation Bull., **4**:67–71, 1957.
6. BILLINGHAM, R. E.; BRENT, L.; and MEDAWAR, P. B. Actively Acquired Tolerance of Foreign Cells. Nature, **172**:603–6, 1953.
7. ———. Acquired Tolerance of Tissue Homografts. Ann. New York Acad. Sc., **59**:409–15, 1955.
8. ———. Quantitative Studies on Tissue Transplantation Immunity. III. Actively Acquired Tolerance. Phil. Trans. Roy. Soc., London, s.B, **239**:357–414, 1956.
9. BILLINGHAM, R. E.; BRENT, L.; MEDAWAR, P. B.; and SPARROW, E. M. Quantitative Studies on Tissue Transplantation Immunity. I. The Survival Times of Skin Homografts Exchanged between Members of Different Inbred Strains of Mice. Proc. Roy. Soc., London, s.B. **143**:43–58, 1954.
10. BILLINGHAM, R. E., and LAMPKIN, G. H. Further Studies in Tissue Homotransplantation in Cattle. J. Embryol. Exper. Morphol., **5**:351–67, 1957.
11. BOOTH, P. B.; PLAUT, G.; JAMES, J. D.; IKIN, E. W.; MOORES, P.; SANGER, R.; and RACE, R. R. Blood Chimerism in a Pair of Twins. Brit. M. J., **1**:1456–58, 1957.
12. BRAMBELL, F. W. R. Immunity and Development. Adv. Science, **46**:1–11, 1955.
13. BRYAN, C. R., and MILLER, W. J. Interaction between Alleles Affecting Cellular Antigens Following a Species Cross in Columbidae. Proc. Nat. Acad. Sc., **39**: 412–16, 1953.
14. CANNON, J. A.; TERASAKI, P. I.; and LONGMIRE, W. P. Unexpected Manifestations of Induced Tolerance to Skin Homografts in the Chicken. Ann. New York Acad. Sc., **73**:862–68, 1958.
15. COHEN, C. Occurrence of Three Red Blood Cell Antigens in Rabbit as the Result of Interaction of Two Genes. Science, **123**:935–36, 1956.
16. COUNCE, S.; SMITH, P.; BARTH, R.; and SNELL, G. D. Strong and Weak Histocompatibility Gene Differences in Mice and Their Role in the Rejection of Homografts of Tumors and Skin. Ann. Surgery, **144**: 198–204, 1956.
17. DANFORTH, C. H., and FOSTER, F. Skin Transplantation as a Means of Studying Genetic and Endocrine Factors in the Fowl. J. Exper. Zool., **52**:443–70, 1929.
18. DUNSFORD, I.; BOWLEY, C. C.; HUTCHINSON, A. M.; THOMPSON, J. S.; SANGER, R.; and RACE, R. R. A Human Blood-group Chimera. Brit. M. J., **2**:81, 1953.
19. EICHWALD, E. J., and SILMSER, C. R. Communication. Transplantation Bull., **2**: 148–49, 1955.
20. EICHWALD, E. J.; SILMSER, C. R.; and CHRISTENSEN, P. F. Communication. Transplantation Bull., **2**:57, 1955.
21. FINERTY, J. C. Parabiosis in Physiological Studies. Physiol. Rev., **32**:277–302, 1952.
22. FORD, C. E.; HAMERTON, J. L.; BARNES, D. W. H.; and LOUTIT, J. F. Cytological Identification of Radiation-chimaeras. Nature, London, **177**:452–54, 1956.

23. FOX, A. S. Genetics of Tissue Specificity. Ann. New York Acad. Sc., **73**:611–34, 1958.
24. GENGOZIAN, N., and MAKINODAN, T. Antibody Response of Lethally X-irradiated Mice Treated with Rat Bone Marrow. J. Immunol., **77**:430–36, 1956.
25. GENGOZIAN, N.; MAKINODAN, T.; CONGDON, C. C.; and OWEN, R. D. The Immune Status of Long-term Survivors of Lethally X-irradiated Mice Protected with Isologous, Homologous, or Heterologous Bone Marrow. Proc. Nat. Acad. Sc., **44**:560–65, 1958.
26. GORER, P. A. Some Recent Work on Tumor Immunity. Adv. Cancer Research, **4**:149–86, 1956.
27. GORER, P. A.; LYMAN, S.; and SNELL, G. D. Studies on the Genetic and Antigenic Basis of Tumour Transplantation. Proc. Roy. Soc., London, s.B, **135**:499–505, 1948.
28. HASEK, M. Embryonic Parabiosis and Related Problems of Immunological Tolerance. Transplantation Bull., **4**:113–15, 1957.
29. HILDEMANN, W. H., and OWEN, R. D. Histocompatibility Genetics of Scale Transplantation. Transplantation Bull., **3**:132–34, 1956.
30. HOECKER, G. Genetic Mechanisms in Tissue Transplantation in the Mouse. Cold Spring Harbor Symp. Quant. Biol., **21**:355–62, 1956.
31. HOECKER, G.; COUNCE, S.; and SMITH, P. The Antigens Determined by the H-2 Locus: A Rhesus-like System in the Mouse. Proc. Nat. Acad. Sc., **40**:1040–51, 1954.
32. IRWIN, M. R., and COLE, L. J. Immunogenetic Studies of Species and of Species Hybrids in Doves, and the Separation of Species-specific Substances in the Backcross. J. Exper. Zool., **73**:85–108, 1936.
33. KOLLER, P. C. Discussion of paper by T. Makinodan. J. Cell. & Comp. Physiol., **50** (Suppl. 1):345–46, 1957.
34. KOPROWSKI, H.; THEIS, G.; and LOVE, R. Immunological Tolerance in Tumour Studies. Adaptation of Ascites Tumours to Homologous or Heterologous Hosts. Proc. Roy. Soc., London, s.B, **146**:37–58, 1956.
35. LEVINE, P. The Influence of the ABO System on Rh Hemolytic Disease. Human Biol., **30**:14–28, 1958.
36. LEVINE, P. E.; ROBINSON, E.; CELANO, M.; BRIGGS, O.; and FALKINBURG, L. Gene Interaction Resulting in Suppression of Blood Group Substance B. Blood, **10**:1100–1108, 1955.
37. LINDSLEY, D. L.; ODELL, T. T.; and TAUSCHE, F. G. Implantation of Functional Erythropoietic Elements Following Total-body Irradiation. Proc. Soc. Exper. Biol. & Med., **90**:512–15, 1955.
38. LITTLE, C. C. A Possible Mendelian Explanation for a Type of Inheritance Apparently Non-Mendelian in Nature. Science, **40**:904–6, 1914.
39. ———. Factors Influencing the Growth of a Transplantable Tumor in Mice. J. Exper. Zool., **31**:307–26, 1920.
40. ———. The Genetics of Tumor Transplantation. *In:* G. D. SNELL (ed.), Biology of the Laboratory Mouse, pp. 279–309. Philadelphia: The Blakiston Co., 1941.
41. ———. Genetics and the Cancer Problem. *In:* L. C. Dunn (ed.), Genetics in the 20th Century, pp. 431–72. New York: The Macmillan Co., 1950.
42. LITTLE, C. C., and JOHNSON, B. W. The Inheritance of Susceptibility to Implants of Splenic Tissue in Mice. Proc. Soc. Exper. Biol. & Med., **19**:163–67, 1922.
43. LITTLE, C. C., and STRONG, L. C. Genetic Studies on the Transplantation of Two Adenocarcinomata. J. Exper. Zool., **41**:93–114, 1924.
44. LITTLE, C. C., and TYZZER, E. E. Further Studies on Inheritance of Susceptibility to a Transplantable Tumor of Japanese Waltzing Mice. J. Med. Research, **33**:393–425, 1916.
45. LOEB, L., and WRIGHT, S. Transplantation and Individuality Differentials in Inbred Families of Guinea Pigs. Am. J. Path., **3**: 251–83, 1927.
46. MAKINODAN, T. Immunology of Bone Marrow Transplantation. J. Cell. & Comp. Physiol., **50** (Suppl. 1):327–51, 1957.
47. MARGOLIN, P. An Exception to the Mutual Exclusion of the Ciliary Antigens in *Paramecium aurelia*. Genetics, **41**:685–99, 1956.
48. MEDAWAR, P. B. A Second Study of the Behaviour and Fate of Skin Homografts in Rabbits. J. Anat., **79**:157–76, 1945.
49. ———. Immunity to Homologous Grafted Skin. I. The Suppression of Cell Division in Skin Grafts Transplanted to Immunized Animals. Brit. J. Exper. Path., **27**:9–14, 1946.

50. ———. Introductory Remarks. Proc. Roy. Soc., London, s.B, **146**:1–8, 1956.
51. ———. The Uniqueness of the Individual. London: Methuen & Co., 1957.
52. MITCHISON, N. A. Passive Transfer of Transplantation Immunity. Nature, **171**: 267–68, 1953.
53. NICHOLAS, J. W.; JENKINS, W. J.; and MARSH, W. L. Human Blood Chimeras. A Study of Surviving Twins. Brit. M. J., **1**: 1458–60, 1957.
54. NOWELL, P. C.; COLE, L. J.; HABERMEYER, J. G.; and ROAN, P. L. Growth and Continued Function of Rat Marrow Cells in X-irradiated Mice. Cancer Research, **16**:258–61, 1956.
55. OWEN, R. D. Immunogenetic Consequences of Vascular Anastomoses between Bovine Twins. Science, **102**:400–402, 1945.
56. ———. Immunological Tolerance. Fed. Proc., **16**:581–91, 1957.
57. OWEN, R. D.; WOOD, H. R.; FOORD, A. G.; STURGEON, P.; and BALDWIN, L. G. Evidence for Actively Acquired Tolerance to Rh Antigens. Proc. Nat. Acad. Sc., **40**:420–24, 1954.
58. PANTELOURIS, E. M. Protection of Foetuses against Radiation Injury. Nature, **181**: 563–64, 1958.
59. PONTECORVO, G. The Genetics of Somatic Cells. J. M. Educ. (in press).
60. PREHN, R. T., and MAIN, J. M. A Comparison between Heterozygous and Homozygous Skin Homografts. J. Nat. Cancer Inst., **15**:191–94, 1954.
61. ———. Number of Mouse Histocompatibility Genes Involved in Skin Grafting from Strain BALB/cAn to Strain DBA/2. *Ibid.*, **20**:207–9, 1958.
62. RENDEL, J. Further Studies of Some Antigenic Characters of Sheep Blood Determined by Epistatic Action of Genes. Acta agr. Scandinav., **7**:224–59, 1957.
63. ROGERS, B. O. The Genetics of Skin Homotransplantation in the Human. Ann. New York Acad. Sc., **64**:741–66, 1957.
64. SCHOENE, G. Die Heteroplastische und Homoplastische Transplantationen. Berlin: J. Springer, 1912.
65. SCHWARTZ, E. E.; UPTON, A. C.; and CONGDON, C. C. A Fatal Reaction Caused by Implantation of Adult Parental Spleen Tissue in Irradiated F_1 Mice. Proc. Soc. Exper. Biol. & Med., **96**:797–800, 1957.
66. SHORT, B. F., and SOBEY, W. R. The Effect of Sex on Skin Grafts within Inbred Lines of Mice. Transplantation Bull., **4**:110–11, 1957.
67. SNELL, G. D. Methods for the Study of Histocompatibility Genes. J. Genetics, **49**: 87–103, 1948.
68. ———. Enhancement and Inhibition of the Growth of Tumor Homiotransplants by Pretreatment of the Hosts with Various Preparations of Normal and Tumor Tissue. J. Nat. Cancer Inst., **13**:719–29, 1952.
69. ———. The Enhancing Effect (or Actively Acquired Tolerance) and the Histocompatibility-2 Locus in the Mouse. *Ibid.*, **15**:665–75, 1954.
70. SNELL, G. D.; SMITH, P.; and GABRIELSON, F. Analysis of the Histocompatibility-2 Locus in the Mouse. J. Nat. Cancer Inst., **14**:457–80, 1953.
71. SNELL, G. D.; WHEELER, N.; and AARON, M. A New Method of Typing Inbred Strains of Mice for Histocompatibility Antigens. Transplantation Bull., **4**:18–21, 1957.
72. STORMONT, C. Acquisition of the J Substance by the Bovine Erythrocyte. Proc. Nat. Acad. Sc., **35**:232–37, 1949.
73. ———. An Example of a Recessive Blood Group in Sheep. Genetics, **36**:577–78, 1951.
74. TOOLAN, H. W. Growth of Human Tumors in Cortisone-treated Laboratory Animals: the Possibility of Obtaining Permanently Transplantable Human Tumors. Cancer Research, **13**:389–94, 1953.
75. TRENTIN, J. J. Induced Tolerance and 'Homologous Disease' in X-irradiated Mice Protected with Homologous Bone Marrow. Proc. Soc. Exper. Biol. & Med., **96**:139–44, 1957.
76. TYZZER, E. E. A Study of Inheritance in Mice with Reference to Their Susceptibility to Transplantable Tumors. J. Med. Research, **21**:519, 1909.
77. UPHOFF, D. E. Genetic Factors Influencing Irradiation Protection by Bone Marrow. I. The F_1 Hybrid Effect. J. Nat. Cancer Inst., **19**:123–30, 1957.
78. VOS, O.; DAVIDS, J. A. G.; WEYZEN, W. W. H.; and VAN BEKKUM, D. W. Evidence for the Cellular Hypothesis in Radiation Protection by Bone Marrow Cells. Acta physiol. pharmacol. neerl., **4**:482–86, 1956.

Discussion*

W. R. SOBEY
University of Sydney, Sydney, Australia

Regarding the remarks of Dr. Owen on the failure of male to female homografts, Dr. Short and myself felt as he does that no more than a "sex effect" had been demonstrated, and references in the literature to a Y chromosome gene were premature. The sex effect is different in different inbred lines of mice. In C57, male to female grafts are rejected early, usually within 14 days, whereas in the DBA line the grafts may persist longer than 120 days. By making reciprocal crosses of such lines and grafting male to female within such crosses we were able to show that the degree of the homograft reaction was associated with the Y chromosome, i.e., a Y chromosome originating from C57 caused a rapid rejection of the graft, whereas one from DBA allowed the graft to persist.

* Following paper by Dr. Owen.

The Inheritance of "Vascular Hemophilia" A New and Interesting Problem in Human Genetics*

JOHN B. GRAHAM, M.D.†
Dept. of Pathology, University of North Carolina, Chapel Hill, N.C.

Last week I attempted to locate the earliest literary reference to a problem of blood coagulation. While reading the translation by Hartmann and Guenther of Paul Morawitz' 1905 monograph on blood coagulation,[1] I noticed that both Aristotle and Hippocrates were credited with having considered the problem. However, Aristotle was a mere 4th-century (B.C.) man and Hippocrates a 5th-century man. It occurred to me that Homer, a 10th-century poet, might provide a more ancient text containing more fundamental insights, and in the Odyssey[2] I found what I was seeking.

You recall that, when Odysseus returned from his travels, he re-asserted his authority by slaying the large group of "free-loaders" who had attached themselves to the court at Ithaca during his absence. Then, as an additional turn of the screw, he made the women of the court clean the banquet hall before he killed them for, as Homer put it, "their secret bussing and cuddling with these brave gallants". Homer describes the tidying-up scene this way.

> The women now came in with dreadful wailing and floods of tears. First they had to carry out the dead bodies, and lay them along the courtyard wall, packed close together under the gallery. Odysseus gave them directions and let them waste no time—they had to obey. Then they cleaned up the tables and seats with sponges and water. *Telemachos and the two men scraped over the floor with shovels, and the women cleared out the scrapings.*" (Italics mine.)

The scrapings, of course, were clotted blood.

When I pointed out this passage to a perceptive friend who has been impressed by the twists and turns of blood clotting doctrine and the contentiousness of the workers, he remarked that he saw profound symbolism in the fact that the earliest known literary reference links blood clotting and a shovel. "The quotation," he continued, "has a contemporary ring; it sounds like a newspaper account of charwomen cleaning an auditorium after one of the Federation's Inter-Society Sessions on Blood Coagulation."

In 1955, I attempted to analyze from the genetic standpoint a portion of the enormous literature on blood coagulation (11). It was pointed out that the blood clotting mechanism was a particularly nice physiological system to study from the genetic standpoint, since all the abnormal phenotypes have similar symptoms, i.e., poor hemostasis or hemorrhagic disease. This 1955 account was modernized somewhat and, together with a new section on the inheritance of severe multiple deficiency states, published in the proceedings of the 1956 Inter-

* The investigations of the author have been supported by research grants H-1333 and H-3140 of the National Heart Institute, Public Health Service.

† Professor of Pathology.

[1] P. Morawitz, The Chemistry of Blood Coagulation, 1905. Trans. by R. C. Hartmann and P. F. Guenther. Springfield, Illinois: Charles C Thomas, 1958.

[2] *The Odyssey*, Trans. by W. H. D. Rouse. New York: Mentor Books, 1950.

national Symposium on Hemophilia (12). Those interested in the genetic aspects of afibrinogenemia, Factor V deficiency, classic hemophilia, Christmas disease, the Stuart defect, or multiple deficiencies are urged to refer to these and other recent reviews (7, 26).

The information presently available concerning the genetic control of hemostasis cannot be summarized in a few minutes. Rather than attempting a superficial general summary, I shall try to analyze a problem concerning the antihemophilic factor (AHF), the clotting factor deficient in classic human hemophilia. This symposium seems an appropriate place for such an analysis, because it will be along genetic lines. My remarks will be concerned with the problems raised by a recently recognized hemorrhagic diathesis known as "vascular hemophilia." I hope to show that this "new" disorder exemplifies certain genetic principles which, while not "new" to geneticists, are generally not taken into consideration by blood coagulationists.

THE PROBLEM OF "VASCULAR HEMOPHILIA"

As you probably know, classic human hemophilia is conceived of as due to physiological deficiency of an essential clotting factor (AHF) and transmitted as a sex-linked recessive characteristic (6). Human hemophilia is in a class almost by itself, because there is an inherited hemorrhagic disorder in dogs which appears identical with it (13). There is no question but that the canine variety of the disorder is sex-linked and recessive (16).

It is generally recognized that the condition known as "vascular hemophilia" is inherited differently than classic hemophilia. To attempt an explanation, I shall have to weave together three strands. These strands will be referred to as "mild hemophilia," "pseudo-hemophilia," and "vascular hemophilia."

"*Mild hemophilia.*"—In the past 5 years, it has become apparent that the genetic control of the plasma AHF level is more complex than had been thought. Much new information about this matter has accumulated, largely as a result of the development of highly specific, semi-quantitative assay procedures for this clotting factor (5, 14, 22, 31).

The first clear indication that AHF level is not always controlled either by the classic hemophilia gene or by its normal allele was the report from our laboratory in 1953 of a mild form of hemophilia (15). We presumed at the time that the gene responsible for the reduction in AHF was allelic with the well recognized locus on the X chromosome. It should be emphasized that allelomorphism was a tentative suggestion. There was no direct evidence then, and there is none now, that the two forms of hemophilia are in fact allelic. Linkage data with respect to some common marker such as color blindness are not available, and no pedigrees segregating for both types of hemophilia have been described. The two types of hemophilia have in common, however, an important feature—reduction in the plasma level of the same clotting factor, AHF. The segregation of "mild hemophilia" in the human kindred described appeared consistent with incompletely recessive sex-linkage, and allelomorphism seemed to be the simplest hypothesis, as Haldane had suggested many years earlier (17). From the time allelism was first proposed, however, workers in this field have suspected that the notion was incorrect. For example, it has been difficult to understand why some of the female carriers of "mild hemophilia" have had clear reductions in plasma AHF levels while other carriers of this form of the disease and the carriers of severe classic hemophilia have not (4, 15, 27, 32).

The evidence that mild hemophilia is sex-linked is statistical and had been accepted, because sex linkage could not be rejected. The crucial test concerned the four sons of two of the five men with reduced levels of AHF. All sons were said to be clinically normal; two of the sons were available for testing and had AHF levels in the normal range, but the other two were overseas. (See Addenda for follow-up on this

point.) Also, all five of the daughters of the affected men who could be classified, either had affected sons or had reduced levels of AHF. The progeny of the thirteen females presumed to be carriers appeared equally divided between normal males, affected males, carrier females, and unclassified, but probably normal, females. The crucial test for sex linkage, of course, lay with the progeny of the affected males (Table 1). With the distribution: two normal males, two unknown males, five carrier females, and seven unknown females, a sex-linkage hypothesis could not be rejected. If "carrier" females and "affected" males are assumed to be similarly heterozygous for an autosomal dominant trait and scored "affected," an autosomal dominant hypothesis can be rejected because of the absence of affected men and normal women ($\chi^2 = 7$, $P < .01$). However, autosomal dominance cannot be rejected if as many as two of the seven unestablished females are, in reality, normal. Of course, the finding of a single affected son from an affected father rejects sex-linkage outright.

What I am trying to communicate is this: The hypothesis that "mild hemophilia" is transmitted by a gene on the X chromosome has never been considered entirely satisfactory either from the genetic or physiologic standpoint. The kindred from which the hypothesis originated was small, and a few females shifted from one cell to another, or an affected son from an affected father would cause sex-linkage to be abandoned. Interestingly, no one to my knowledge has attempted to confirm or reject this idea. It has been accepted without question and carried along in the literature for 5 years.

"Pseudohemophilia".—The term "pseudohemophilia" has probably been used at one time or another to represent every hemorrhagic disease which has been mistakenly considered identical with hemophilia. When the difference has been established, the new arrival has been, temporarily at least, designated "pseudohemophilia." I am going to use the term in a way which Humpty-Dumpty would applaud, i.e., to mean "just what I choose it to mean—neither more nor less."[3] "Pseudohemophilia" will refer to the bleeding disorder segregating in a kindred described by MacFarlane and Simpkiss in 1954 (25). This is a very interesting study for several reasons and not widely appreciated. The kindred described is fairly large; many people in it were studied by many modern methods (although the actual data were not recorded satisfactorily) and, very important, a *single* abnormality is apparently being transmitted.

Eleven of the eighteen affected members of the MacFarlane kindred, with a relatively mild bleeding diathesis and prolonged bleeding time, were tested and found normal with respect to clotting time, clot retraction, platelet numbers, morphology, and function, the various known "clotting factors," fibrinogen, thrombin generation, thromboplastin generation, prothrombin consumption, and prothrombin time. The bleeding time prolongation was transmitted by both mothers and fathers to both sons and daughters. Eight parents, abnormal by test, had 21 children who were tested. The children were distributed as: three normal males, four affected males, nine normal females, and five affected females (Table 2). This is not a significant deviation from a 1:1:1:1 ratio ($\chi^2 = 1.11$, $P > .20$), and there was male-male transmission. It appears, therefore, that "pseudohemophilia," *meaning a prolonged bleeding time without any other defect detectable by a wide variety of tests*, is transmitted in this kindred as a simple autosomal dominant.

TABLE 1

THE CHILDREN OF FIVE MEN AFFECTED WITH "MILD HEMOPHILIA"

(Graham *et al.*)

MALES			FEMALES		
Normal	Affected	Unknown	Normal	Carriers	Unknown
2	0	2	0	5	7

[3] Lewis Carroll, *Through the Looking Glass*, p. 94. New York: Random House Edition, 1946.

"*Vascular hemophilia*".—An abstract describing an unusual hemorrhagic disorder appeared in the minutes of a national meeting in the United States in 1953 (2, 3). It described prolonged bleeding time and reduction of the plasma AHF in two patients, a man and a girl. (To my knowledge, complete case reports have never been published on these patients.) This syndrome has attracted much attention, because the time-honored axiom regarding hemophilia (or AHF deficiency) has been that the clotting time is prolonged but the bleeding time is normal.

Shortly after the first report in 1953, workers in Paris (23) reported a similar syndrome in a small French girl. In 1955, two other affected girls were reported from Holland and Canada (8, 9). The Dutch patient, in particular, had severe symptoms; her similarly affected sister had died of cerebral hemorrhage.

TABLE 2

THE CLASSIFIED CHILDREN OF EIGHT PARENTS TESTED AND FOUND AFFECTED WITH "PSEUDOHEMOPHILIA"

(MacFarlane and Simpkiss)

	Children			
Affected	Males		Females	
parents	Normal	Affected	Normal	Affected
6 Fathers	1	4	6	4
2 Mothers	2	0	3	1
8 Parents	3	4	9	5

Then in rapid succession in June, September, and October, 1956, seventeen other cases were reported. The mother of a girl described in June by workers from Chicago (38) had an AHF level of 75 per cent of normal, and the father had a level of 128 per cent; the Chicago workers suggested that they were describing an autosomal recessive form of AHF deficiency.

In September, a group working in New York (37) reported seven cases, four males and three females. Their material was not completely homogeneous, since six children had AHF levels of less than 1 per cent, and the seventh had 100 per cent. Also, the bleeding times of the patients ranged from a minimum one of 6–8 minutes (barely abnormal) to several greater than 15 minutes. Nevertheless, the fact that they had studied three small girls with clearly reduced AHF levels, whose fathers were not overt hemophiliacs, suggested that there might be an autosomal locus involved in regulating plasma AHF level. The New York group reported that only one parent had had a history suggestive of excessive bleeding and that studies on him were normal, thus reinforcing the recessive notion. They examined the capillaries of the bulbar conjunctivae and the nailbeds with the slit-lamp microscope and reported that the capillaries were tortuous in all seven patients. Great significance was attached to the capillary abnormality, and the name "vascular hemophilia" was suggested for the syndrome.

In October, a group working in Seattle reported five small kindreds totaling 138 persons and containing 34 individuals in whom an increased bleeding tendency was assumed from "either the history, or clinical or laboratory findings" (29). It is difficult to understand why all these patients were considered to have the same syndrome. Four persons who were extensively studied and scored as affected had bleeding times in the normal range. Four others (two of the ones with normal bleeding times and two others) had AHF levels above the lower limit of the normal range. Thus, the conclusion that one half of the persons in four of the families were affected with an identical disorder must be regarded with some skepticism. This paper was important chiefly because it reported two crucial rejections of sex linkage (father-son transmissions) as the mode of inheritance of the condition among tested individuals. Most of the extensively studied patients in this series were female (six of nine) and only three of seven patients examined had visible capillary abnormalities. Although it appeared to them that capillary tortuosity was not crucial in the pathogenesis of the condition, the authors continued to use the name "vascular hemophilia."

In 1957, four more papers on "vascular

hemophilia" appeared, three from a pair of workers in Cologne, Germany (1, 20, 21). The German workers reported two small kindreds and two sporadic cases of "vascular hemophilia." One kindred (20) showed an interesting feature. The grandfather had a bleeding time at the upper limit of normal and reduced AHF level. The father, his son, had both a clearly prolonged bleeding time and reduced AHF. Two grandsons were normal, but the grand-daughter showed a normal bleeding time and reduced AHF. The other kindred (21) showed transmission of both prolonged bleeding time and reduced AHF from two affected brothers to some of their children, one a male-male transmission.

At this point, in mid-1957, it was clear that a "new" inherited hemorrhagic diathesis had been delineated from the hazy "unclassified" category. It was being variously called "vascular hemophilia" (29, 37), "pseudohemophilia B" (38), and "angiohemophilia" (1, 21). There was general agreement that it was not inherited as a sex-linked trait and that reduction in plasma AHF and prolonged bleeding time were the key features. Clotting time and prothrombin consumption were usually normal to only slightly abnormal. Platelet counts, clot retraction, platelet thromboplastic activity, and capillary fragility were normal. There might or might not be abnormalities in the structure of the capillaries by microscopy. This seemed no longer essential.

The severity of the syndrome varied widely, ranging from mild epistaxis and easy bruising to severe hemarthrosis and even fatal hemorrhage. Clinical severity appeared to be a function of both the *length of the bleeding time* and the amount of *reduction in plasma AHF*. As might have been expected, the bleeding times varied widely between different studies, more so than did the AHF assays. Even with the assays, however, there was considerable variation within and between families.

Late in October, 1957, a very complete study appeared from Sweden (30). This study is outstanding because the material is large, six different families having been studied extensively, and uniform methods were used. As I hope to show in a few minutes, the Swedish study has provided information which allows the formulation of a genetic hypothesis reconciling "mild hemophilia," "pseudohemophilia," and "vascular hemophilia."

In the Swedish report both bleeding time and AHF level are recorded on many members of two of the six families, and AHF levels alone are given on large numbers in the other four families. The method of ascertainment in this study, which has been the same in all other kindreds reported in the literature, is important to note, because of the bias it introduces. All families have been discovered through a severely affected individual, and there have been very few other severely affected persons discovered. Widespread study of the six Swedish kindreds uncovered only three other severely affected individuals. One was a parent of the proband in Family E; another was a very distant relative of the proband in Family A, and the third was a dead sibling of the proband of Family D.

Table 3 contains data which illustrate the relationship between the bleeding times and AHF levels of the severely affected patients and the parents probably transmitting the reduction in AHF. It will be noted that the parent in every instance has a reduced level of AHF which is, nevertheless, considerably higher than that of his child. Also note that the affected children had very long bleeding times, while the parents' bleeding times were increased little if at all. These nine severely affected patients came from very small sibships which (including the patients themselves) totaled only thirteen persons, and only one of the severely affected persons (IV-7 of Family E) has had a descendant (V-15). (This last was a father-son transmission of both AHF deficiency and prolonged bleeding time, decisively disposing of any simple sex-linkage hypothesis for either trait.)

If one considers the possibility of transmission of the severe bleeding disorder as

a simple autosomal unit characteristic, the problem is difficult. It becomes that of understanding how a presumably heterozygous parent with a mild to moderate reduction in AHF and a normal bleeding time and mated to a normal person can produce a child with a much more severe reduction in AHF and much longer bleeding time. It is particularly difficult to consider the bleeding time prolongation a secondary effect of the AHF deficiency, because the classic hemophiliac with almost no plasma AHF has a normal bleeding time.

The distribution of the abnormal values, which have been italicized in the table, suggests that, in the sibships of the transmitting parents, prolonged bleeding time and reduction in AHF have been segregating independently—i.e., the aunts and uncles of the probands have had one or the other defect but not both. This idea is bolstered by data on the children of certain of the siblings of the proband's mother in Family B. Four of these siblings with reduced AHF had ten children. Seven of their ten children had normal AHF levels, and three had reduced AHF levels, but *all had normal bleeding times*. Unfortunately, it has not been possible to analyze the inheritance of long bleeding times alone through the children of persons with elevated bleeding times and normal AHF's. There were three suitable parents in Family A (IV-7, IV-10, and IV-14) and one in Family B (III-14), but either they had no children or their children have not been studied.

TABLE 3

RELATIONSHIP BETWEEN THE SEVERELY AFFECTED CHILD AND THE PARENT PROBABLY TRANSMITTING THE REDUCED AHF LEVEL

(Nilsson *et al.*)

	AFFECTED CHILD (ALL SEVERELY AFFECTED)				TRANSMITTING PARENT				
FAMILY	No. in pedigree	Sex	Bleeding time (min.)	Plasma AHF, per cent of normal	No. in pedigree	Sex	Bleeding time, (min.)	Plasma AHF, per cent of normal	Severity of symptoms
A	V, 6	F	>60	3	IV, 6	F	6	62	None
B	IV, 2	F	>60	4	III, 4	F	3½	35	None
	IV, 3	F	>60	4					
C	IV, 1	F	>60	6–16	III, 2	F	Normal	48	None
D	IV, 4	F	>60	5	III, 3	F	Normal	45	None
	IV, 5	F	dead, not studied						
E	V, 15	M	30	15	IV, 7	M	4–10	21	Severe
	IV, 7	M	4–10	21	III, 4	F	?	49	None
F	IV, 4	M	240	5	III, 5	F	Normal	25	None

I think insight may be obtained by comparing the tests from all the members of the two more thoroughly studied Swedish families who show either a reduction of AHF below 62 per cent or a bleeding time greater than 6 minutes. (I have chosen these discriminants, because the highest AHF level of a parent presumably transmitting the disorder in Table 3 was 62 per cent, and the upper range of normal for the bleeding time is said to be 5 minutes).

It can be seen in Table 4 that, aside from the probands and their fathers, there were seven persons from Family A and ten from Family B who had either prolonged bleeding times or reduced AHF or both. Five had prolonged bleeding times alone,

Table 5 shows that persons of both sexes with AHF levels below 62 per cent transmitted levels of less than 62 per cent to

one half of their children of both sexes. Thus, the Swedish material strongly suggests that the mildly reduced AHF level is transmitted as an autosomal dominant.

These findings tend to suggest a hypothesis more complex, but more reasonable, than any yet proposed—that the hereditary clinical syndrome "vascular hemophilia" results from the combined effects of two abnormal genes. One of them causes reduction in AHF; the other causes increase in bleeding time. The AHF locus is autosomal and dominant, as the Swedish material clearly shows, but variably expressive. The AHF reduction caused by this gene is almost never as severe as that caused by the classic hemophilia gene carried on the X chromosome, but the method of ascertainment has

TABLE 4

THE MEMBERS OF FAMILIES A AND B WITH ABNORMAL AHF LEVEL OR BLEEDING TIME

(Nilsson *et al.*)

PEDIGREE NO.	RELATIONSHIP TO PROBAND	BLEEDING TIME, (MIN.)	AHF LEVEL, PER CENT NORMAL
	Family "A"		
V, 6	Proband	*>60*	*3*
IV, 6	Mother	*6*	*62*
IV, 8	Mother's brother	*2½*	*55*
IV, 15	Mother's brother	*2*	*47*
IV, 7	Mother's sister	*15*	100
IV, 10	Mother's brother	*8*	88
IV, 14	Mother's sister	*7*	95
III, 4	Distant maternal relative	*>60*	*5–10*
IV, 5	Father	1	78
	Family "B"		
IV, 2	Proband	*>60*	*4*
IV, 3	Proband	*>60*	*5*
III, 4	Mother	*3½*	*35*
III, 6	Mother's brother	1	*20*
III, 12	Mother's sister	*3½*	*52*
III, 15	Mother's brother	1	*20*
III, 16	Mother's brother	*4–6½*	*21*
IV, 5	Mother's sister's daughter	*1½*	*44*
IV, 10	Mother's brother's son	*2½*	*59*
IV, 13	Mother's brother's daughter	*2½*	*36*
III, 14	Mother's sister	*7–10*	95
IV, 1	Father's sister's child	*7½*	75
III, 3	Father	*3*	85

With Prolonged bl. time : 5
With reduced (<62%) AHF: 10
With both : 2

Abnormal values are in italics.

TABLE 5

INHERITANCE OF REDUCED AHF LEVEL IN SIX SWEDISH FAMILIES

(Nilsson *et al.*)

AFFECTED PARENTS	CHILDREN: Males Normal	Males Affected	Females Normal	Females Affected	χ^2	P (per cent)
9 Fathers	2	7	10	7	3.18	> 5
6 Mothers	2	2	3	6	.32	>50
15 Parents	4	9	13	13	1.30	>20

selected probands in whom the gene has had a particularly severe effect. As was pointed out earlier, MacFarlane's pedigree of "pseudohemophilia" indicates that simple prolongation of bleeding time may be transmitted as an autosomal dominant. MacFarlane also pointed out that the bleeding time prolongation was highly variable among affected individuals and within affected individuals at different times.

The possibility, suggested by the genetic analysis, that bleeding time and AHF level are unrelated phenomena, is also suggested by independent biochemical evidence (30). It has been shown by the Swedish workers that certain sub-fractions of Cohn's Fraction I, *without AHF activity*, will shorten the bleeding time of patients severely affected with "vascular hemophilia" *without changing the clotting time or AHF level!* This suggests that a previously unrecognized plasma factor is reduced in "vascular hemophilia" and possibly also in MacFarlane's kindred (25), causing the prolonged bleeding times.

How does this hypothesis about "vascular hemophilia" relate to "mild hemophilia" of the allegedly sex-linked variety, when it is known that bleeding times were normal in the "mild hemophilia" kindred? (I know that these bleeding times were normal, because I did them myself!) It will be recalled that the sex linkage hypothesis was based on a very small amount of information, and that a change of one or two persons into another category would have caused rejection of the hypothesis. "Vascular hemophilia," "pseudohemophilia," and "mild allelic hemophilia" can be reconciled if it is assumed:

a) That "mild hemophilia" is actually autosomally transmitted, the original report having been in error, and that in this kindred "pseudohemophilia," meaning prolonged bleeding time, was *not* segregating.

b) That "vascular hemophilia" occurs in families segregating for both "mild hemophilia" and "pseudohemophilia." The persons in whom both defects coincide and have strong effects are the ones with clinically severe "vascular hemophilia," i.e., the probands.

This hypothesis would explain two puzzling features of "vascular hemophilia," (*a*) why so few persons in a family segregating for "vascular hemophilia" are severely affected and (*b*) why an excess of females with this disease (25 females to seventeen males at last count) has been reported.

The scarcity of severely affected patients in a kindred, once ascertained, would be due to the low probability of being doubly heterozygous. Only one person in four would be expected to be doubly heterozygous in a sibship in which two rare autosomal dominants are segregating independently. It is probably also necessary that one or both of the abnormal genes have an unusually strong effect in order to produce the full-blown syndrome. (For example, the mother of the proband in Family A of the Swedish material would be scored abnormal on both counts, with a 6-minute bleeding time and 62 per cent AHF. Yet she had no symptoms.)

The excess of females in published reports is probably related to two factors. (*a*) When a male with severe bleeding symptoms and reduction in AHF is observed, he is usually labeled a "hemophiliac" and a bleeding time is not done. If his bleeding time is tested and proves to be abnormal, it is usually dismissed as an artifact. When a female with a hemorrhagic tendency and reduced AHF is observed, she becomes a research project; all tests are carried out on her, and she is correctly diagnosed. It is probable that our diagnostic files contain a sizable number of male patients with the syndrome lying unnoticed among the hemophiliacs. (*b*) The discovery of a "new" disease always makes alert observers re-examine cases previously set aside as obscure. Generally speaking, such a spurt of activity will result in the examination of more women than men, since females predominate among the obscure "unclassified" bleeders in the diagnostic file.

All the data necessary to test the two-factor thesis of "vascular hemophilia" are

not yet available. The following experiments should provide a crucial test:

1. Administration of Fraction 1, *lacking AHF activity*, to the affected patients in MacFarlane's pedigree of "pseudohemophilia" to see whether this bleeding time defect is the same as that in the Swedish material. This might also be done with the relatives of the Swedish patients with long bleeding times but normal AHF levels.

2. Re-study of the original pedigree of "mild hemophilia" to see whether exceptions to sex linkage (male-to-male transmission of AHF deficiency) can be found. (See Addenda.)

3. Determination of bleeding times of the children in the Swedish families whose parents had prolonged bleeding times but normal AHF levels.

If these three studies proved consistent with the two-factor thesis, I would consider it established as probably correct.

Now, if in "mild hemophilia" and "vascular hemophilia" the plasma AHF reduction results from an abnormal autosomal gene, what does this suggest about the control of plasma AHF level? The fact that loci on both the X chromosome and an autosome can effect a reduction in the AHF level suggests, as one possibility, that at least two loci are concerned with AHF synthesis. This may mean that AHF is synthesized in two or more steps. Such a possibility should occasion no surprise, because AHF is thought to be a large protein molecule. You will recall that AHF synthesis is optimal whenever one or more normal sex-linked alleles are present in the genome (normal hemizygote = heterozygote = normal homozygote). This suggests that the step under X chromosome control may be one in which a chemically simple but physiologically crucial thing happens to the molecule, such as the acquiring of an active site. It seems unlikely that at this step there are complex chemical reactions requiring large amounts and varieties of reactants. The reverse argument probably can be made for the autosomal locus. There are, of course, other ways to explain how AHF synthesis can be affected by two different loci. It will be very interesting to study a patient who is homozygous at the autosomal locus for AHF-deficiency, because those homozygous at the X chromosome locus are not more severely affected than the hemizygotes (16) and the heterozygotes are usually normal.

Now, the hypothesis that "vascular hemophilia" represents the summation along a final, common hemostatic pathway of double heterozygosity for two dominant autosomal genes, each of which alone has a slight effect on hemostasis, has greater generality in the field of hemorrhagic diseases then appears at first glance. There are already other similar syndromes on the horizon. For example, there is a hemorrhagic disorder present at high frequency among the Åland islanders (an isolated population living off the coast of Finland). It has been suggested recently that these people have a mild AHF deficiency coupled with a qualitative defect in platelets (19). Furthermore, reports are beginning to appear of patients with both mild PTC-deficiency (Christmas disease) and prolonged bleeding times (1, 18, 39, 40).

Many factors both plasmatic and cellular are now known to be necessary for normal hemostasis. As one abnormal phenotype after another is discovered, it is becoming apparent that these factors are under genetic control. The fact that the AHF level can be affected by mutation at more than one genetic locus can probably be extended to the other factors, known and unknown. This implies that there probably are many still unrecognized mutant genes in the population with *individually small* effects on clotting factors. Since the frequency of a gene in the population will be inversely proportional to the selective effects against it, genes with mild effects should be present at higher frequencies than genes with severe effects, such as the classic hemophilia gene. It would not be surprising, therefore, if certain other obscure hemorrhagic disorders proved to be mild double defects or a combination of severe and mild defects. It seems probable

that the specification of these disorders will require genetic analysis in addition to conventional blood clotting studies.

From the practical standpoint, a patient with hemorrhagic symptoms more pronounced than some well recognized laboratory procedure appears abnormal and should be considered exceptional and suspected of suffering from an additional unrecognized defect. The converse is also possible. A common laboratory procedure affected by many variables such as the thromboplastin generation test (4) or the partial thromboplastin time (22, 36) may show a defect more marked than clinical symptoms would suggest. In such a case one should suspect that the patient may have, in addition to his primary defect, reduction in one of the factors which affect laboratory tests but not intravascular hemostasis, such as the Hageman factor (10, 24, 28, 33–35). The fact that a syndrome is hereditary does not necessarily imply that inheritance is simple or unifactorial.

SUMMARY

1. The history of the evolvement of a "new" hemorrhagic disorder, "vascular hemophilia," is described. It is pointed out that this syndrome raises interesting problems in the interpretation of the genetic control of AHF synthesis.

2. The fact that the AHF level can be affected by abnormal genes on either the X chromosome or an autosome suggests, among other possibilities, that synthesis of the AHF molecule occurs in at least two steps.

3. It is hypothecated that "vascular hemophilia" represents double heterozygosity for a dominant autosomal gene affecting AHF synthesis and another dominant autosome affecting bleeding time. The crucial experiments with respect to this hypothesis remain to be done, and several are outlined.

4. It is suggested that others of the obscure hemorrhagic disorders may represent double heterozygosity for mild defects and that genetic analysis of the families of such patients is a very important part of the study of these disorders.

ADDENDA

1. Mr. Bennie Ward reinvestigated, in the summer of 1958, that portion of the North Carolina kindred of "mild hemophilia" crucial from the genetic standpoint, i.e., the affected male with three sons. Bleeding times on all four were determined again and found normal, and a reduced AHF level (29 per cent) was confirmed in the father. (The mother's AHF had previously been found to be 100 per cent.) Two sons had AHF levels greater than 100 per cent, while the third one, previously untested, had a level of 51 per cent. We expect to replicate these studies several more times before trying to decide whether this represents male-male transmission of "mild hemophilia."

2. Dr. J. H. Renwick of the Galton Laboratory kindly (and diplomatically) pointed out that I overlooked pleiotropism as an explanation for "vascular hemophilia." I think this possibility did not occur to me, because of the biochemical evidence that two factors are deficient in "vascular hemophilia" and of previous conditioning along the lines of the one gene-one enzyme hypothesis. If "vascular hemophilia" does, in fact, result from a single pleiotropic autosomal gene, this mutation is a very interesting one affecting two chemically different products.

The decision between these two possibilities appears to require widespread and careful studies of several large families with "vascular hemophilia." If pleiotropism is the explanation, additional severely affected patients should be found widely scattered through the kindreds. Also, one should find children with low AHF values whose parents show only prolonged bleeding times and children with prolonged bleeding times whose parents show only low AHF values. If the "two-gene" notion is correct, severely affected persons who are not close kin of a proband should be very rare. Also, parents

with one defect alone should not transmit the other.

3. It was reported at the VIIth Congress of the International Society of Hematology (Rome, Sept., 1958) by Nilsson, Blombäck, and Blomäck that plasma transfused from a "classic hemophiliac" to a "vascular hemophiliac" corrects the bleeding time without affecting the AHF level. This settles rather decisively the question whether AHF itself is related to bleeding time.

REFERENCES

1. ACHENBACH, W., and KLESPER, R. Angiohämophilie A und B. *Folio Haemat.* (new series), **1**:251–67, 1957.
2. ALEXANDER, B., and GOLDSTEIN, R. Dual Hemostatic Defect in Pseudohemophilia. J. Clin. Investigation, **32**:551, 1953.
3. BIGELOW, F. S. Serotonin Activity in Blood: Measurement in Normal Subjects and in Patients with Thrombocythemia Hemorrhagica and Hemorrhagic States. J. Lab. Clin. Med., **23**:759–73, 1954.
4. BIGGS, R., and DOUGLAS, A. S. The Thromboplastin Generation Test. J. Clin. Path., **6**:23–29, 1953.
5. BIGGS, R.; EVELING, J.; and RICHARDS, G. The Assay of Antihemophilic-Globulin Activity. Brit. J. Haematol., **1**:20–34, 1955.
6. BRINKHOUS, K. M., and GRAHAM, J. B. Hemophilia and the Hemophilioid States. Blood, **9**:254–57, 1954.
7. BRINKHOUS, K. M.; LANGDELL, R. D.; and WAGNER, R. H. Hemostatic Disorders: Hemophilia and the Hemophilioid Diseases. Ann. Rev. Med., **9**:159–90, 1958.
8. VAN CREVELD, S.; JOKDAN, F. L. J.; PUNT, K.; and VEDER, H. A. Deficiency of Antihemophilic Factor in a Woman Combined with a Disturbance in Vascular Function. Acta Med. Scandinav., **151**:382–89, 1955.
9. DARTE, J. M. Defect of A-hemophilic Globulin in von Willebrand's Disease. Am. J. Dis. Child., **90**:561, 1955.
10. FRICK, P. G., and HAGEN, P. S. Severe Coagulation Defect without Hemorrhagic Symptoms Caused by a Deficiency of the Fifth Plasma Thromboplastin Component. J. Lab. & Clin. Med., **47**:592–601, 1956.
11. GRAHAM, J. B. The Biochemical Genetics of Blood Coagulation. Am. J. Human Genetics, **8**:63–79, 1956.
12. ———. Genetic Problems; Hemophilic and Allied Diseases. *In:* K. M. BRINKHOUS (ed.), Hemophilia and Hemophilioid Diseases, pp. 137–62. Chapel Hill, N.C.: University of N.C. Press, 1957.
13. GRAHAM, J. B.; BUCKWALTER, J. A.; HARTLEY, L. J.; and BRINKHOUS, K. M. Canine Hemophilia. J. Exper. Med., **90**:97–111, 1949.
14. GRAHAM, J. B.; COLLINS, D. L., JR.; GODWIN, I. D.; and BRINKHOUS, K. M. Assay of Plasma Antihemophilic Activity in Normal, Heterozygous (Hemophilia), and Prothrombinopenic Dogs. Proc. Soc. Exper. Biol. & Med., **77**:294–96, 1951.
15. GRAHAM, J. B.; MCLENDON, W. W.; and BRINKHOUS, K. M. Mild Hemophilia: an Allelic Form of the Disease. Am. J. M. Sc., **225**:46–53, 1953.
16. GRAHAM, J. B.; SWANTON, M. C.; and BRINKHOUS, K. M. Ten Years' Experience Breeding Hemophilic Dogs (in preparation).
17. HALDANE, J. B. S. The Rate of Spontaneous Mutation of a Human Gene. J. Genetics, **31**:317–26, 1935.
18. INGRAM, G. I. C. Observations in a Case of Multiple Haemostatic Defect. Brit. J. Haematol., **2**:180–93, 1956.
19. JÜRGENS, R.; LEHMANN, W.; WEGELIUS, O.; ERIKSON, A. F.; and HIEPLER, E. Über den mangel an antihämophilem Globulin (AHG, Faktor VIII) bei der Ältädischen hereditären Thrombopathie (von Willebrand-Jürgens), p. 125. VI Congress de la societe Europeene d'Hematologie, 1957.
20. KLESPER, R., and ACHENBACH, W. Über die Sogenannte Pseudohämophilie. Klin. Wchnschr., **35**:1007–13, 1957.
21. ———. Über eine Familie mit Angiohämophilie A (vaskuläre Hämophilie). Thromb. et Diath. Haem., **1**:223–33, 1957.
22. LANGDELL, R. D.; WAGNER, R. H.; and BRINKHOUS, K. M. Effect of Antihemophilic Factor on One-Stage Clotting Tests. J. Lab. & Clin. Med., **21**:637–47, 1953.
23. LARRIEU, M. J., and SOULIER, J. P. Déficit en facteur antihémophilique à chez une fille associé à un trouble du saignement. Rev. Hemat., **3**:361–70, 1953.
24. LARRIEU, M. J.; SOULIER, J. P., and CULOT, Y. Déficit en facteur Hageman. Sang, **28**: 152–59, 1957.
25. MACFARLANE, J. C. W., and SIMPKISS, M. J. The Investigation of a Large Family

with von Willebrand's Disease. Arch. Dis. Children, **29**:483–87, 1954.

26. MacFarlane, R. G. Blood Coagulation with Particular Reference to the Early Stages. Physiol. Rev., **36**:479–502, 1956.
27. Margolius, A., Jr., and Ratnoff, O. D. A Laboratory Study of the Carrier State in Classic Hemophilia. J. Clin. Investigation, **35**:1316–23, 1956.
28. ———. Observations on the Hereditary Nature of Hageman Trait. Blood, **11**:565–69, 1956.
29. Matter, M., Newcomb, T. F.; Melly, A.; and Finch, C. A. Vascular Hemophilia: the Association of a Vascular Defect with a Deficiency of Antihemophilic Globulin. Am. J. M. Sc., **232**:421–33, 1956.
30. Nilsson, I. M.; Blombäck, M.; and von Franken, I. On an Inherited Autosomal Hemorrhagic Diathesis with Antihemophilic Globulin (AHG) Deficiency and Prolonged Bleeding Time. Acta Med. Scandinav., **159**: 35–57, 1957.
31. Pitney, W. R. The Assay of Antihaemophilic Globulin (AHG) in Plasma. Brit. J. Haematol., **2**:250–64, 1956.
32. Ramot, B.; Angelopoulos, B.; and Singer, K. Variable Manifestations of Plasma Thromboplastin Component Deficiency. J. Lab. & Clin. Med., **46**:80–88, 1955.
33. Ramot, B.; Singer, K.; Heeler, P.; and Zimmerman, H. J. Hageman Factor (HF) Deficiency. Blood, **11**:745–50, 1956.
34. Ratnoff, O. D. Hageman Factor and Hageman Trait. *In:* K. M. Brinkhous (ed.), Hemophilia and Hemophilioid Diseases, pp. 124–29. Chapel Hill, N.C.: University of N.C. Press, 1957.
35. Ratnoff, O. D., and Colopy, J. E. A Familial Hemorrhagic Trait Associated with a Deficiency of a Clot Promoting Fraction of Plasma. J. Clin. Investigation, **34**:602–13, 1955.
36. Rodman, N. F., Jr.; Barrow, E. M.; and Graham, J. B. Diagnosis and Control of the Hemophilioid States with the Partial Thromboplastin Time (PTT) Test. Am. J. Clin. Path., **29**:525–38, 1958.
37. Schulman, I.; Smith, C. E.; Erlandson, M.; Fort, E.; and Lee, R. E. Vascular Hemophilia. A Familial Hemorrhagic Disease in Males and Females Characterized by Combined Antihemophilic Globulin Deficiency and Vascular Abnormality. Pediatrics, **18**:347–61, 1956.
38. Singer, K., and Ramot, B. Pseudohemophilia Type B. Arch. Int. Med., **97**:715–25, 1956.
39. Sjølin, K. E., and Videbaek, A. Christmas Factor Deficiency and Decreased Capillary Resistance in a Female with Haemorrhagic Diathesis. Dan. M. Bull., 85–87, 1956.
40. Soulier, J. P., and Larrieu, M. J. Déficit en facteur antihemophilique B avec allongement du temps de saignement. Sang, **28**: 138–41, 1957.

Panel: Selective Factors in the ABO Polymorphism

WILLIAM C. BOYD
Boston University, School of Medicine, Boston, Mass.

INTRODUCTION

Ladies and gentlemen, you see before you a reformed character. I formerly believed, and even used it as an argument for the use of blood groups in physical anthropology, that the blood group genes were selectively neutral. In taking this attitude I was doubtless influenced by certain physical anthropologists who had been arguing that human classification should be based on "non-adaptive" characters, as being the least likely to be changed by the action of evolutionary forces. I have since come to believe that there probably are no neutral genes and that classification has to be based on genes which have been affected by the forces which bring about racial differentiation, mainly mutation and selection. (In fairness it should be remarked that some of the anthropologists also changed their minds.) I am, therefore, a former believer in neutral genes who has recanted, and it accordingly gives me great pleasure to introduce this panel of papers on agencies possibly affecting blood group gene frequencies.

A Possible Example of the Action of Selection in Human Blood Groups?

WILLIAM C. BOYD
Boston University, School of Medicine, Boston Mass.

Some years ago my wife and I devised methods for determining the blood groups of mummies. The methods employed were serological and would be of interest mainly to serologists. In any case they have been fully described (3). We felt fully as skeptical as anybody else about claims, which might be considered to border almost on the fantastic, to have detected blood group antigens in material as much as 5,000 years old, and employed all the controls we could think of. Our confidence in the methods was increased by the fact that we found we could regularly determine the blood group of dried muscle taken from individuals of a known blood group, and by the fact that the results on Egyptian and American Indian mummies differed in just the way which would be expected from the present-day blood group distributions of these peoples. Our confidence has been further increased by later findings of organic material, including amino acids, which in general are less stable than blood group substances, in much older material (1). Finally, we observed that we regularly obtained the same results in such work whether we employed blood grouping reagents of human or rabbit origin.

In most American Indian mummies we found either no blood group antigen (leading to a diagnosis of group O) or antigen A, but in a few, including several from Peru (3), a "Big Bend Basket Maker" from Goat Cave, Texas (3), and two specimens excavated by Dr. W. W. Taylor (4) at Coahuila in Mexico, we apparently found antigen B.

We were not particularly surprised by these results at the time, for a number of workers had reported the presence of blood group B in American Indians, and in fact two groups of Indians, the Yámanas of Tierra del Fuego and the Carajas of the Amazon basin, had been reported to have very high frequencies of the B gene. It did not seem surprising, therefore, that some of the older inhabitants of the new world should also have possessed this gene. Since then, however, the situation has completely changed. The finding of B in the Yámanas and Carajas has been shown by retesting to be the result of gross technical error, and analyses of the available Indian data, including one such analysis made by me (2), have shown beyond any doubt that pure blood American Indians do not possess the gene B.

Now this absence of the gene B in the American Indians is in many ways the most surprising thing about them. American Indians are typically Mongoloid, and anthropologists are convinced that their ancestors came across the Bering Strait, some 20,000 to 25,000 years ago, from Asia, where blood group gene B is particularly frequent. It is thus not easy to explain why Indians do not also have B. There have been attempts to explain this, and I have suggested that it is the result of genetic drift or of an initial chance absence of B in the original immigrants. However, both of these explanations necessarily suppose that the numbers of immigrants were extremely small, smaller than linguistic, archeological, and cultural evidence makes probable, and the situation has remained something of a puzzle.

If B has in fact always been absent in

American aborigines, then our finding of B in the new world specimens must be an error, or the B must be the result of white mixture and the material cannot be very old. We are unfortunately not in a position to say anything new about the provenance of the Peruvian material we tested. Some anthropologists have doubted its antiquity. However, we do have new information about the material from Coahuila. Dr. Taylor assures me he was and is convinced that the stratigraphic evidence indicated considerable antiquity, and more recently carbon-14 dating has shown that the material is definitely pre-Columbian, some of it being in fact over 8,000 years old. The possibility of technical error remains, but examination of our original notebooks shows that the tests were conducted with, if anything, rather more than our usual care, and that each of these specimens was tested and retested until the material was used up. Therefore, although we admit in principle that any one of our determinations could be in error, we are not disposed to think that our Coahuila results were especially subject to this risk, particularly not to the extent of leading us falsely to report an antigen in two different specimens from the same site. Furthermore, if technical error were responsible, we should have to suppose that this also affected our work on the Big Bend Basket Maker specimen (3).

I should therefore like to suggest the possibility of another explanation. Let us suppose, for the sake of argument, that the ancestors of the American Indians reached this continent with blood group B, but that some selective force, due to the American environment or to the enforced new diet, or to some cause we do not understand, led to the (relatively) rapid elimination of gene B, so that the gene frequencies decreased, in the course of 20,000 years or so, from values in the neighborhood of 0.3 to 0. Such decrease would not be impossible if B were acted on by a selective agency as strong as some of those suggested by certain of the recent work, particularly if it were not opposed by any compensatory mechanism tending to preserve a balanced polymorphism in regard to the B and O genes (see previous papers in this panel).

If such a selective agency did act, it would explain why the American Indians, in spite of their Asian ancestry, today lack the gene B. It would also explain our finding of B in some American mummies, particularly in the Coahuila material, for we might well suppose that 5,000 years ago the elimination of B was still incomplete. At the same time, the possibility that B had already been largely eliminated might account for our failure to find B in most other American material, especially since most of this material was probably not so old.

It must be admitted that this suggestion is pure speculation, and that it is hard to think of any way to test it. If there is any truth in it, however, it offers a striking example of the action of natural selection in modifying one of the blood group gene frequencies in one part of the world.

One possibility, not indeed of proving this speculation, but of making it somewhat more probable, should be mentioned. According to Dr. Taylor, some tissue from the Coahuila specimens still remains, and is probably available. If retest of this material should again indicate the presence of the B antigen, I for one should then be inclined to put forward the above speculation for serious consideration as a possible explanation for the absence of B in American Indians.

REFERENCES

1. ABELSON, P. H. Some Aspects of Paleobiochemistry. Ann. N.Y. Acad. Sc., **69**:276, 1957.
2. BOYD, W. C. Blood Groups of American Indians. Am. J. Phys. Anthrop., **25**:215, 1939.
3. BOYD, W. C., and BOYD, L. G. Blood Grouping Tests on 300 Mummies. J. Immunol., **32**:307, 1937.
4. TAYLOR, W. W., and BOYD, W. C. Blood Groups of the Prehistoric Indians of Coahuila by Serological Tests of Their Mummified Remains. Year Book Am. Phil. Soc., p. 178, 1943.

Correlations of ABO Blood Groups with Peptic Ulcer, Cancer, and Other Diseases

C. A. CLARKE, M.D., F.R.C.P.*
University of Liverpool, Liverpool, England

The particular problem with which this paper is concerned is whether individuals belonging to different ABO blood groups do or do not differ in their susceptibility to certain common "adult" diseases. The question is not a new one. As long ago as 1921 Buchanan and Higley at the Mayo Clinic investigated the matter (4) and came to the conclusion that there was no association between any of the ABO groups and the diseases which they tested—and these included peptic ulcer, pernicious anemia, and various types of malignant disease.

Interest lay almost dormant for 30 years and was only renewed in 1953 when Aird and his colleagues (2) in England published a paper showing that there was a striking relationship between group A and carcinoma of the stomach in all parts of the country. Other authors have since published similar findings, although there is one large series, from Vienna, where the association has not been found. Aird and his colleagues (1) also investigated three other cancers, those of the colon and rectum, lung, and breast, and obtained negative results. In the same paper they described the biggest association of all, that between peptic ulcer and blood group O. They concluded that an individual who was group O was 35 per cent more prone to develop an ulcer than one who was not O.

Since 1954 a great deal of literature has accumulated. The association between group O and peptic ulcer has been found in many parts of the world—Boston, Iowa, Copenhagen, Oslo, and Vienna, to mention some of them, and it has also been found in Chinese, Japanese, and in Negroes. Usually the association has been greater with duodenal than with gastric ulcer, and it seems probable that there is a real difference between the two diseases. Table 1 shows the results we obtained in Liverpool (1955) and it can be seen that in our series the figures for gastric ulcer do not differ significantly from those for the controls. The controls in this and all the other series I have so far mentioned have been obtained either from grouped blood donors or from over-all grouped hospital populations, excluding those suffering from the disease being investigated. I shall refer to this again later.

TABLE 1

LIVERPOOL DATA (1955)

	O	A	B	AB
Duodenal ulcer: 860	505	263	62	30
Percentages	58.72	30.58	7.21	3.49
Gastric ulcer: 377	185	151	31	10
Percentages	49.07	40.05	8.22	2.65
Controls: 15,377	49.0	39.1	9.4	2.5

* Reader in Medicine.

In 1956 a group of workers in England showed that there was a convincing association between pernicious anemia and blood group A (7).

The next part of the story concerns the secretor character in duodenal ulcer. As is well known, some individuals secrete their ABH antigens in their body fluids and some do not. The character is inherited in a simple mendelian way, secretion being dominant to non-secretion. We thought that there might be physiological differences between those who did and those who did not secrete their antigens and that it might not be entirely fortuitous that the saliva and gastric juice of secretors contained particularly large quantities of these blood group substances. We therefore investigated (1956) the character in duodenal ulcer and found a highly significant association between the disease and non-secretion. Table 2 shows the details of this survey, and this part of the work was entirely carried out by Dr. R. B. McConnell. Table 3 shows the range of the diseases which have been tested for secretor character, and it will be seen that the only significantly abnormal finding is that for duodenal ulcer.

TABLE 2

SECRETION OF ABO BLOOD GROUP ANTIGENS; PERCENTAGES OF NON-SECRETORS (1956)

Duodenal ulcer: 973 patients	36.6 per cent
General population control: 849 patients	24.4 per cent
No heterogeneity for blood group or sex	
Comparison ulcers: Controls χ_1^2	31.64
	$P<0.001$.

Table 4 shows the details of what we may regard as the well established associations between blood groups and adult diseases.

It appeared at first sight as though the various findings could be readily correlated. We surmised that individuals who were group O might be pouring out excess hydrochloric acid in their stomachs and therefore be liable to duodenal ulcer and only rarely develop gastric cancer. On the other hand, with group A, there was the association with gastric carcinoma and pernicious anemia, the latter always and the former often showing achlorhydria. Then there was the knowledge that the secretor substances were mucopolysaccharides and therefore in non-secretors there might be less of the protective mucoid barrier in the stomach.

The first criticism came from Penrose.[1] He thought that the findings might be due to stratification and that it was possible that there might be in the population a strain high in O and high in duodenal ulcer but with no causal connection. He said that before causality could be accepted family studies should be made, and we have investigated up to the present time nearly 400 duodenal ulcer sibships. In all the other series that have been published, the controls have been blood donors or over-all grouped hospital populations, but in our family studies it is the unaffected sibs who act as controls, and they cannot be criticized on the ground that they do not come from the same population as the ulcer population.

TABLE 3

SECRETOR CHARACTER AND DISEASES

	No. tested	Per cent Nonsecretor
Controls	849	24.4
Duodenal ulcer	973	36.6
Gastric ulcer	132	28.0
Aphthous ulcer	156	23.7
Carc. stomach	167	19.2
Carc. cervix	220	28.3
Diabetes mellitus	318	26.1
Asthma	250	23.6

We have analysed the data by the method of Dr. C. A. B. Smith of the Galton Laboratory, London. The principle of Smith's method is to assess in each segregating family the chance of the propositus being O and then to compare the total observed results with the total expected. It must be emphasized that in this type of analysis it is only families which segregate for blood group—some of the sibs being O and some not O—that can be used, and many families there-

[1] Personal communication, 1953.

fore give no information. If we consider the simplest possible case, that of a duodenal ulcer sibship consisting of four individuals, two of whom are group O and two of group A, clearly the chance of the propositus being group O is an even one, and the expected result is therefore scored as 0.5. If, in fact, the propositus is group O, then the observed score is 1, whereas if he is group A, the observed score is zero.

Table 5 illustrates the results in 134 sibships which segregated for the O blood group. It will be seen that the observed score is slightly higher than the expected, but nowhere near significantly so. Nor is significance obtained if the propositus is paired with a sib of her or his own sex. These findings do not contradict the hypothesis that group O predisposes to ulcer, but they give no support to it.

Table 5 also shows the data analysed for secretion and nonsecretion. Again it will be seen that within families an individual who is a nonsecretor is not significantly more likely to have the ulcer than his secretor sib. If, however, the propositus is paired with a sib of the same sex, significance is obtained, but it seems doubtful from the way the work is going whether this will hold with more material.

Since I spoke about this in Copenhagen, there is some additional evidence of interest. Dr. Richard Doll of the Central Middlesex Hospital, London, has recently started a similar sibship investigation in duodenal ulcer, and he has very kindly allowed me to quote his preliminary results.[2] In 63 propositi with a total of 69 sibs he has found no association within families between group O or nonsecretion with the ulcer.

The explanation of these findings is difficult. We are loath to accept the explanation of stratification, since it seems incredible that the same stratification could occur here in the U.S.A., in England, and also in Denmark, Norway, and Portugal. We are therefore driven to consider the possibility, unlikely though it may seem in a disease such as duodenal ulcer, of a maternal factor. If mothers who are group O are more likely than mothers who are group A, B, or AB to produce children who will develop duodenal ulcer irrespective of *their* blood group, the ulcer population would be being bred from a high O strain and would give you the type of result which we have in fact obtained. Such a maternal effect might operate im-

[2] Personal communication, 1958.

TABLE 4

WELL ESTABLISHED ASSOCIATIONS WITH ABO BLOOD GROUPS*

	Duodenal ulcer	Gastric carcinoma	Pernicious anemia
Number of centers reporting	9	13	9
Number of patients analysed	8,272	6,795	1,498
Difference from controls	Group O +16.8 per cent	Group A +10.0 per cent	Group A +13.5 per cent

* From J. A. Fraser Roberts (9).

TABLE 5

ANALYSIS OF DUODENAL ULCER SIBSHIPS*

	Propositus Group O	Propositus nonsecretor
Expected	65.326	44.283
Observed	69	48
Difference	+ 3.674	+ 3.717
S.E.	± 5.577	± 4.705
P	> 0.5	> 0.4

* Method of C. A. B. Smith.

TABLE 6

FUCOSE IN SALIVA*

	No. tested	Mean fucose (μg/ml)	Standard error
O Secretor	23	93	±10.9
O Nonsecretor	23	78	± 6.5
A Secretor	23	86	± 7.5
A Nonsecretor	23	77	± 8.4
B Secretor	17	101	±12.1

* D. A. P. Evans' figures.

munologically or as a behavior difference between O and non-O women. There is something of a parallel if we consider the ABO blood groups of children with erythroblastosis due to anti-D. If in 1938, knowing nothing about rhesus, someone had investigated the ABO groups of erythroblastotic babies, he would have found that they were higher in O than the general population but that this association would not have held in sibships (see paper by Levine in this symposium).

Whether some antigen-antibody reaction occurring *in utero* could possibly cause predisposition to duodenal ulcer seems highly speculative, but any clue is worth pursuing, and we have continued to investigate the problem from both the biochemical and immunological points of view—in spite of our sibship results. The data may be summarized as follows:

1. Peebles Brown and his colleagues in Glasgow (3) have shown by means of augmented histamine test meals that individuals who are group O do not have potentially more hydrochloric acid in their stomachs than those who are not O.

2. Morgan (8) has pointed out that the nonsecretors of ABH are usually secretors of Lewis, and therefore, in terms of total blood group mucopolysaccharide in the stomach, there will be nothing to choose between the secretors and nonsecretors of ABH. One of our team, Dr. Price Evans, is investigating this matter at the moment. The sugar fucose provides a very good index of blood group activity, and Evans has found in his results thus far that there is no significant difference in the amount of fucose in the saliva of secretors and nonsecretors of ABH—in other words, in the nonsecretors of ABH the fucose is coming from the secretion of Lewis (Table 6). Before leaving this point, I should mention that about 8 per cent of the population are known to be nonsecretors of both ABH and Lewis. We wondered therefore, whether duodenal ulcer patients might show an excess of this type of individual, but we have not found this to be so.

3. If the association between duodenal ulcer and nonsecretion is due to a direct effect of the secretor gene, then there might be quantitative differences in antigen titer in the saliva of individuals who are secretors compared with their unaffected secretor sibs—we might expect a lower titer, i.e., less antigen in those who have the ulcer. As far as we have gone (about 70 sib pairs) there is no significant difference in titer between the saliva of the individual with the ulcer and that of the sib without. If this proves to be our final conclusion it will be strong evidence against the possibility that the association between nonsecretion and duodenal ulcer is due to a direct effect of the secretor gene.

4. Coombs has shown, by means of the mixed agglutination technique, that the ABH antigens are present not only on red cells but on many types of surface epithelium as well. Coombs found that antigens were present regardless of whether the individual was a secretor or nonsecretor. We felt that it would be worth while investigating this matter in relation to suspensions of duodenal cells, and one of our team, Dr. Frances Selsnick, an American citizen, is carrying out this work.[2] She is using fluorescein-labeled antibodies as well as Coombs' technique, and, although the results are only tentative, it rather looks as though in the case of duodenal cells the nonsecretors do *not* have antigens on their striated borders, whereas the secretors do. If this proves to be our final view, it would encourage us to explore further the possibility that there might be immunological factors at work in the production of duodenal ulcer. We did at one time postulate that ABH agglutinins in plants might be playing a part—many of the Leguminosae have anti-H or anti-A substances, and the possibility that substances such as soya beans which are used as improvers could be toxic because of their antibody content crossed our minds—but it has been shown that most of these plant antibodies are destroyed by boiling, and therefore this hypothesis rather deteriorates.

[2] Personal communication, 1958.

CONCLUSION

The whole problem, therefore, of the relationship of duodenal ulcer to blood group O remains unsolved. The association does not show up in families, and the biochemical data do not show how the association, if a true one, is exerting its effects. An immunological reaction remains a possible explanation, and we think the blood groups of mothers who have borne children who develop duodenal ulcer are worth investigating.

Nevertheless, the evidence from all parts of the world regarding the association between group O and ulcer, when blood donors are used as controls, is very strong. It is possible that, for some reason, the effect of the O gene is diluted in families because of other genes which predispose to ulcer, and these may be more in evidence in ulcer sibships than in the general population.

ACKNOWLEDGMENTS

We are grateful to the United Liverpool Hospitals Research Committee under the chairmanship of Lord Cohen of Birkenhead and to the Nuffield Foundation for their generous support.

REFERENCES

1. AIRD, I.; BENTALL, H. H.; MEHIGAN, J. A.; and ROBERTS, J. A. F. The Blood Groups in Relation to Peptic Ulceration and Carcinoma of Colon, Rectum, Breast and Bronchus. An Association between the ABO Groups and Peptic Ulceration. Brit. M. J., **2**:315, 1954.
2. AIRD, I.; BENTALL, H. H.; and ROBERTS, J. A. F. A Relationship between Cancer of Stomach and the ABO Group. Brit. M. J., **1**:799, 1953.
3. BROWN, D. A. P.; MELROSE, A. G.; and WALLACE, J. The Blood Groups in Peptic Ulceration. Brit. M. J., **2**:135, 1956.
4. BUCHANAN, J. A., and HIGLEY, E. T. The Relationship of Blood Groups to Disease. Brit. J. Exper. Path., **2**:247, 1921.
5. CLARKE, C. A.; COWAN, W. K.; EDWARDS, J. W.; HOWEL-EVANS, A. W.; MCCONNELL, R. B.; WOODROW, J. C.; and SHEPPARD, P. M. The Relationship of the ABO Blood Groups to Duodenal and Gastric Ulceration. Brit. M. J., **2**:643, 1955.
6. CLARKE, C. A.; EDWARDS, J. W.; HADDOCK, D. R. W.; HOWEL-EVANS, A. W.; MCCONNELL, R. B.; and SHEPPARD, P. M. ABO Blood Groups and Secretor Character in Duodenal Ulcer. Population and Sibship Studies. Brit. M. J., **2**:725, 1956.
7. Collective Series from a Number of Centres. Association between Blood Group A and Pernicious Anaemia. Brit. M. J., **2**:723, 1956.
8. MORGAN, W. T. J., quoted by Levine. *In:* MCCONNELL, R. B. & SHEPPARD, P. M., Discussion on the Secretor Character and Disease. Acta genet., **6**:579, 1956/57.
9. ROBERTS, J. A. F. Blood Groups and Susceptibility to Disease. Brit. J. Prev. Soc. Med., **2**:107, 1957.

Selection in ABO Polymorphism in Japanese Populations

EI MATSUNAGA
Department of Legal Medicine, Sapporo Medical College, Sapporo, Japan

In order to study selection in ABO polymorphism, it is necessary, as the first step, to collect evidence from as many diverse sources as possible. As the second step, an estimation must be made of the selective forces on certain genotypes at different ontogenetic stages. Particular attention must be paid to whether a counterbalancing agent is operating, since it is thought that a constant gene frequency or equilibrium is usually maintained in natural populations. Table 1 summarizes some criteria for recognition of selection in relation to the ABO blood groups at different developmental stages. For clinical interest, selection at a very early stage may perhaps be of less interest than if grown persons of certain genotypes are more susceptible to a malignant disease. From the viewpoint of genetics, however, equal attention must be paid to selection at any stage as long as it is affecting an individual in such a way that, compared with those of the other genotypes, he cannot contribute proportionately to the gene content of the population in the following generation. In this paper, I would like to discuss the general topic using our data from Japanese populations but comparing them with those from American whites.

TABLE 1

CRITERIA FOR RECOGNITION OF SELECTION IN ABO BLOOD GROUPS AT DIFFERENT STAGES

Stages	Genotypically conditioned differences in
Time of fertilization	Fertility of parent, the sex ratio in the children
Intra-uterine	Occurrence of spontaneous abortions
Peri-natal	Occurrence of stillbirth and hemolytic disease
Post-natal and adult life	Resistance or susceptibility to diseases, longevity

The discovery by Levine and his coworkers (11) that hemolytic disease of the newborn is caused by maternal-fetal isoimmunization in the Rh blood group system has made a new epoch in the field of blood group serology, since it has established beyond doubt that the blood groups do have different selective values. This recognition stimulated a search for such selection in other blood group systems, and many reports have appeared pointing out that not only hemolytic disease but also spontaneous abortions can be caused by maternal-fetal ABO incompatibility. The term incompatibility is applied to those instances when the mother is lacking a dominant blood factor that is present in the fetus. In ABO blood groups, the following instances are incompatible: mother O and fetus A or B, mother A and fetus B or AB, and mother B and fetus A or AB. These matings are in contrast to those compatible matings in which the blood factors of the child and of the mother are identical or in which the mother carries the dominant factor.

A direct approach to the estimation of the extent of this selection could be made by family investigations in which exact reproductive differences in reciprocal mating

types are examined. During the period of from 1953 to 1954 an extensive field study was carried out in our laboratory on 1429 families of laborers from mining companies in two mining town areas in Hokkaido, namely, Ashibetsu and Kohnomae. From each couple blood specimens were collected for routine tests of the blood groups of ABO and MN systems. Examination of the $Rh_0(D)$ antigen was omitted, because the incidence of Rh_0 positive persons is assumed to be more than 99 per cent in the Japanese population, and it was considered that, for comparison of fertility between reciprocal mating types with respect to the Rh system, a much larger body of data would be necessary. Careful marital histories were taken, particular attention was paid to the number of living children, and all pregnancies including spontaneous abortions were recorded. It was found that at the time of this investigation there was scarcely any effective control over family size in the population samples of these localities.

Since the distributions of each blood group and parental combination showed no sign of heterogeneity between the two areas in both ABO and MN systems, the data were pooled for analysis of differential fertility in reciprocal mating types. In Tables 2 and 3 are presented the summaries of the results of the comparison between two major mating groups, i.e., compatible and incompatible. There was no indication for selection due to incompatibility in the MN system, whereas in ABO blood groups a highly significant difference was demonstrated between the two mating groups in two respects. In the incompatible mating group, the frequencies of abortions and of childless couples were distinctly higher than in the compatible mating group. The mean number of pregnancies per woman did not show a significant difference, although the frequency of infertile couples was higher in the incompatible group than in the compatible one, the difference being significant at the 5 per cent level. This may be explained by the possibility that selection might be at work at the earliest period of pregnancy under conditions unknown to

TABLE 2

FERTILITY OF COMPATIBLE AND INCOMPATIBLE MATING GROUPS IN MN BLOOD TYPES

	Compatible	Incompatible
No. matings	885	544
Proportion of couples as yet infertile	9.83 per cent	9.38 per cent
Mean no. pregnancies (per woman)	3.210 ± 0.077	3.173 ± 0.094
Proportion of abortions (per pregnancy)	12.28 per cent	12.69 per cent
Proportion of childless couples	13.56 per cent	13.24 per cent
Mean no. living children (per woman)	2.403 ± 0.084	2.430 ± 0.077
Mean no. living children (per pregnancy)	0.749	0.766

TABLE 3

DIFFERENTIAL FERTILITY BETWEEN COMPATIBLE AND INCOMPATIBLE MATING GROUPS IN ABO BLOOD GROUPS

	Compatible	Incompatible	Test for significance
No. matings	812	617	
Proportion of couples as yet infertile	8.13 per cent	11.67 per cent	$0.05>P>0.02$
Mean no. pregnancies (per woman)	3.250 ± 0.080	3.125 ± 0.091	$0.30>P>0.10$
Proportion of abortions (per pregnancy)	10.34 per cent	15.30 per cent	$P<0.001$
Proportion of childless couples	9.85 per cent	18.15 per cent	$P<0.001$
Mean no. living children (per woman)	2.596 ± 0.064	2.173 ± 0.070	$P<0.001$
Mean no. living children (per pregnancy)	0.799	0.696	

the mother. Further, as expected, a highly significant reduction in the mean number of living children per woman has been demonstrated in the incompatible mating group compared with that in the compatible mating group. Under the assumption that fertilization occurred with equal frequency in both mating groups, it was calculated that the mortality rate of those children incompatible with the mothers, whether the cause of death be abortion, stillbirth, or hemolytic disease, amounts to 21 per cent in this population.

The magnitude of this powerful selection pressure is not very surprising when we consider that the intra-uterine and peri-natal period is the most critical one from the point of view of natural selection. To compare the present results of a Japanese population with those of Europeans or American whites, it is necessary to consider both genetic and environmental factors influencing the fetal and neonatal morbidity. The incidence of maternal-fetal incompatibility in any blood group system depends on the genetic composition of the population. Table 4 illustrates the comparison of the occurrence of hemolytic disease due to incompatibility both in ABO and Rh systems in American whites and Japanese with special reference to the incidence of the incompatible matings in these populations. The frequency of Rh incompatible matings amounts to about 13 per cent in American whites, while it is only about 0.5 per cent in Japanese. In accordance with these figures, the incidence of hemolytic disease by Rh incompatibility in Japanese is much lower than that in American whites. On the other hand, the incidence of ABO incompatible matings is about 44 per cent in Japanese, whereas in American whites it is about 36 per cent. Corresponding to this difference, the occurrence of neonatal deaths by ABO incompatibility is higher in Japanese than in American whites.

In connection with the above differences in the genetic composition of the two populations, it should be taken into consideration that there is an interaction between Rh incompatibility and ABO incompatibility. Levine (9) drew attention to the fact that mothers of children with hemolytic disease due to Rh isoimmunization were more often compatibly mated in regard to ABO system than were unselected women. This finding was substantiated by later workers on this field, and it has been explained by assuming that ABO incompatibility protects from Rh isoimmunization in the case when the pregnancy is incompatible for both the Rh and ABO systems (Race and Sanger [18]). In a study of a Swedish population by Grubb and Sjöstedt (5), it was found that in a series of marriages with two or more pregnancies terminating in intra-uterine death for unknown reasons the frequency of ABO incompatible matings was about 50 per cent in the mating class Rh-positive × Rh-positive, while in those involving at least one Rh-negative partner only about 20 per cent of the matings were ABO incompatible, and the discrepancies were statistically significant. Therefore, in

TABLE 4

INCIDENCE OF HEMOLYTIC DISEASE OF THE NEWBORN IN AMERICAN WHITES AND JAPANESE

POPULATION	FREQUENCY OF INCOMPATIBLE MATINGS WITH RESPECT TO ABO system (per cent)	FREQUENCY OF INCOMPATIBLE MATINGS WITH RESPECT TO Rh system (per cent)	INCIDENCE OF HEMOLYTIC DISEASES IN FULL-TERM PREGNANCIES DUE TO ABO incompatibility	INCIDENCE OF HEMOLYTIC DISEASES IN FULL-TERM PREGNANCIES DUE TO Rh incompatibility
American Whites	36	13	1:1500*	1:150*
Japanese	44	0.5	1:200†	1:6600~1:16000†

* After Levine (10).
† After Kihara (8).

a population such as Japanese where almost all matings are of Rh-positive × Rh-positive, it would be not so difficult to detect the effect of the selection due to ABO incompatibility, while in European populations or American whites the over-all effect of such selection could perhaps be reduced to a certain extent by the action of Rh incompatibility.

Here, it may be worth while to call attention to the fact that infant death from ABO incompatibility could begin at the first pregnancy in contrast to cases caused by Rh incompatibility where the mother will usually be more intensively sensitized with succeeding pregnancies. Table 5 represents the distribution of living children in the compatible and incompatible mating groups in our data. The frequencies of childless couples, as has been stated, differ significantly between the two mating groups, but the frequencies of those couples with one to five living children, respectively, show no significant differences, while the frequency of couples with more than six children is about 7 per cent in the compatible matings—and this figure is more than twice that in the incompatible one, the difference being again significant. Childless couples may be the result of ABO incompatibility, while one-child sterility might be produced by Rh incompatibility. The conceivable explanations for this finding may be made in the following ways: from the frequent fetal deaths caused by ABO incompatibility, it is likely that the antibody-producing power of the A and B antigens is much stronger than that of Rh, so that isoimmunization in the former case is sufficient to cause infant death in the first pregnancy. Moreover, it is possible that the normal anti-A or anti-B in mother's serum would affect the fetus early in pregnancy before the mother has been sensitized. Evidence for this hypothesis is, however, still lacking, and further research is needed.

So far we have discussed the subject from genetical and serological points of view. It is evident, however, that incompatibility is a necessary condition for such selection but not a sufficient one. Since incompatibility does not always cause abortions or hemolytic disease, there must be certain yet unknown factors which, when added to the incompatibility, cause the diseases. Apart from these factors, there are several agents affecting the magnitude of this selection. Reed (19) suggested that pre-natal selection can often be demonstrated when the mean parity exceeds two but that the degree of selection may vary with parity. As Glass (4) demonstrated in American whites regarding Rh blood group system, it can be expected that, in a population where effective control over family size exists and a pattern of very small size of family has been established, the differential fertility between ABO compatible and incompatible matings may be changed even in the opposite direction, owing to the so-called overcompensation. Moreover, general living conditions, especially those concern-

TABLE 5

DISTRIBUTION OF LIVING CHILDREN IN COMPATIBLE AND INCOMPATIBLE MATINGS

No. children	Compatible matings		Incompatible matings		χ^2 value with Yate's correction
	No.	Per cent	No.	Per cent	
0	80	9.85	112	18.15	20.06 ($P<0.001$)
1	161	19.83	130	21.07	0.16
2	198	24.38	147	23.82	0.02
3	170	20.94	102	16.53	2.78
4	84	10.34	63	10.21	0.01
5	62	7.64	43	6.97	0.12
6~10	57	7.02	20	3.24	8.17 ($P<0.01$)
Totals:	812	100.00	617	99.99	31.32 ($P<0.01$)

ing the health of the population studied, have an appreciable effect. The frequency of stillbirths including spontaneous abortions occurring after the fourth month of pregnancy that have been registered legally in the City of Sapporo in Hokkaido from 1949 to 1953 amounts to 14.5 per cent of both livebirths and stillbirths (Inouye [6]). In the population of the mining town areas investigated by us, the living conditions seemed to be much worse than in the city of Sapporo. These agents must have influenced to a large extent the magnitude of selection by ABO incompatibility in that population. However, I would like to note here that the situations are of course quite different according to locality and seem to be rapidly changing in Japan during the last few years.

Going back to the discussion of the subject from the viewpoint of population genetics, the action of selection due to maternal-fetal incompatibility is directed against children of heterozygous genotypes. The selection must work, therefore, to depress the gene ratio of that allele which is less common in the population. In the case of ABO polymorphism, it follows that, for the Japanese population as well as for populations in most parts of the world, selection would work so as to decrease the gene frequency of A and B and to increase the frequency of O. As a result, the gene ratio would no more be balanced and it would change from generation to generation until the frequencies of both dominant genes would be maintained at very low level by recurrent mutation. In view of the present higher frequencies of these alleles in human populations, it should be surmised that other selective forces must be operating in order to compensate the losses of the heterozygotes or the losses of A and B genes.

According to Fisher's theory for maintenance of a balanced polymorphism, a stable equilibrium of gene frequencies depends on opposed forces and can be produced (*a*) when a heterozygote has a selective advantage over both homozygotes or (*b*) in certain conditions when there is an interaction between two or more groups of alleles in such a way as to alter one another's selective values. An unstable equilibrium is produced, on the contrary, when a heterozygote is at a disadvantage compared with both homozygotes. These general principles can now be applied to the consideration of the possible compensatory mechanism for the maintenance of genetic equilibrium in ABO polymorphism. The first attempt to seek for such compensatory mechanism should be made, therefore, by examination whether in ABO blood groups heterozygotes may have selective advantage over homozygotes. Heterozygous individuals may have larger fertility or viability than homozygous individuals in post-natal life. It is also conceivable that compensating selection may be operating during the pre-natal period in such a way that heterozygous fetuses may have an advantage over homozygous fetuses when there is no incompatibility.

In the above data of the mining town areas there was an indication that group O fathers may be less fertile than heterozygotes. Within compatible mating groups, the mean number of pregnancies and of living children in those matings with group O fathers was significantly lower than those in the matings in which neither parent belongs to O (Table 6). Since the frequencies of abortions are almost equal in both mating subgroups, the difference in the mean number of children is obviously due to the difference in the mean number of pregnancies. The genotypes of the children from the matings, in which the father belongs to O, are AO, BO, and OO, while from those matings in which neither parent belongs to O, children of AA, BB, and AB genotypes can be born, in addition to AO, BO, and OO. The proportion of these two mating subgroups is 54:46, so that this differential fertility might be of sufficient magnitude to compensate for the losses of A and B genes eliminated through incompatibility. It is necessary, however, to test whether compensation of this form can restore the genetic equilibrium, whenever it is destroyed by incompatibility. According to a personal

communication from Dr. Kimura, who has recently analyzed this point mathematically, it was proved that, assuming the counterbalance between the effect of decreased fertility of O father and that of incompatibility, an equilibrium is reached at certain gene frequencies; but it is not a stable one. Compensation of such a form cannot lead to a balanced polymorphism.

Another form of compensation by heterosis may now be put forward in the following model; assuming only pre-natal selection, a heterozygous fetus of certain genotypes, say AO, has a disadvantage of i compared with homozygotes when pregnancy is incompatible, while it has a selective advantage of h when the pregnancy is compatible. In a random mating population, the frequencies of AO fetuses that are compatible and incompatible with the mothers are $\overline{AO}_{comp}$ and $\overline{AO}_{incomp}$, respectively. Then the following equation formula should be held for a balance of gene ratio:

$$h \cdot \overline{AO}_{comp} = i \cdot \overline{AO}_{incomp} .$$

If the value of $h \cdot \overline{AO}_{comp}$ is larger than that of $i \cdot \overline{AO}_{incomp}$, it would presumably lead to a stable equilibrium, whereas it would be changed to an unstable one if the former value were smaller than the latter. Since the heterozygotes of both AO and BO cannot, unfortunately, be recognized serologically on a wide scale, it would be difficult to collect data to test this hypothesis. However, regarding the AB heterozygote, a direct approach to the analysis of this point can be made. The frequency of AB in Japanese population is about 10 per cent, so that it is much higher than that in Europeans. The proportion of those AB children incompatible with the mother in the total AB is exceedingly high; in Japanese population approximately 78 per cent of AB fetuses are incompatible with the mother. If the selection pressure due to incompatibility is of a magnitude of 0.21, as calculated in the population of the mining town areas, then the value of h, calculated from the above formula, should be 0.74 for a neutral equilibrium. On the other hand, the relative fitness in viability of AB heterozygotes compared with AA and BB homozygotes can be calculated when we obtain sufficient data from a homogeneous population regarding the number of children of different genotypes born from AB × AB matings. From the literature of Japanese family investigations which have been carried out on more than three thousand matings at the Department of Legal Medicine of both Kanazawa Medical College and Tokyo University directed by Dr. Furuhata, 33 such matings were collected (Table 7). Of 83 children born, eleven were AA, eighteen BB, and 54 AB. The AB:(AA + BB) ratio was 1.86, which indicated a significant excess of the AB children. The observed value of h amounts to 0.86, which is higher than that value for the equilibrium: The differ-

TABLE 6

DIFFERENTIAL FERTILITY BETWEEN TWO SUBGROUPS OF COMPATIBLE MATING GROUPS

	Mother Father A×A B×B AB×AB AB×A AB×B	Mother Father A×O B×O AB×O O×O	Test for significance
No. matings	373 (46 per cent)	439 (54 per cent)	
Proportion of couples as yet infertile	7.51 per cent	8.66 per cent	0.70>P>0.50
Mean no. pregnancies (per woman)	3.472±0.125	3.062±0.101	0.02>P>0.01
Proportion of abortions (per pregnancy)	10.19 per cent	10.49 per cent	
Proportion of childless couples	9.92 per cent	9.79 per cent	
Mean no. living children (per woman)	2.743±0.101	2.472±0.082	0.05>P>0.02
Mean no. living children (per pregnancy)	0.790	0.807	

ence was not significant, but it suggests that the compensation may probably be leading in this way to a stable equilibrium.

From what has been said, it will be clear that the counterbalancing selection against that by incompatibility is most probably operating in a sufficient magnitude through the mechanism of heterosis. On the other hand, there is some evidence for compensatory mechanism through a selective interaction between ABO and other blood group systems. As has been mentioned, the interaction of ABO and Rh incompatibility has been established, and the same thing is very probably happening in many other blood group systems which can cause hemolytic disease or abortions by the same mechanism of isoimmunization. As a result of such interaction, selection against heterozygotes in a certain blood group system can be at the same time selection for heterozygotes in another system. As Woolf (24) pointed out, ABO incompatibility is working as a protection against hemolytic disease due to Rh incompatibility, so that heterozygotes are standing at a selective advantage over homozygotes when the pregnancy is simultaneously incompatible regarding Rh system. In European populations or American whites, where the Rh-negative persons are at relatively high frequency, it will be especially interesting to see whether the compensation of this form is of sufficient magnitude to be a significant factor in producing a stable equilibrium in the ABO polymorphism.

So far we have discussed the selection operating in pre-natal and early life in connection with the strong natural selection caused by maternal-fetal incompatibility. During the last few years, evidence is accumulating from different parts of the world for associations between blood groups and a variety of adult diseases. Among these, the most overwhelming is the evidence for association with three diseases, i.e., duodenal ulcer, gastric ulcer, and carcinoma of the stomach. In the first two, persons of group O are at a disadvantage; in the last one, persons of group A. Here I may quote the result of preliminary nature of recent investigations being carried out by Dr. Uetake and others in Tokyo, where a large body of data can easily be collected. The data concern only peptic ulcer and gastric carcinoma, which were obtained from the records of the Hospital of Cancer Research Institute covering the period 1946 to 1956. Diagnostic criteria depend in most cases on histological examinations but in a few cases on roentgenological or other clinical findings. The data were evaluated by comparing the observed blood group frequencies with those of about 2000 pupils of primary and middle schools. The statistical significance of the differences in blood group frequencies occurring between the patients and controls was tested by the χ^2 method.

TABLE 7

FREQUENCY OF AB CHILDREN FROM AB × AB MATINGS

INVESTIGATOR	No. MATINGS	No. CHILDREN AA	BB	AB	Totals
Furuhata (1932)	14	4	7	22	33
Sugishita (1935)	2		1	3	4
Suzuki (1936)	1		1	2	3
Imamura (1937)	2		1	4	5
Hayashida (1944)	3		2	4	6
Arai (1944)	2		2	4	6
Kaneda (1951)	1			3	3
Matsunaga (1954)	8	7	4	12	23
Totals	33	11	18	54	83

$\frac{AB}{AB + BB} = 1.86$; χ^2 value with Yate's correction = 6.94, $P < 0.01$.

In Table 8 are illustrated the basic data showing the results of the O:A:B:AB and O:A comparisons. No significant difference was found between sexes in any series of the data. In consistence with the results reported from the other parts of the world, a significant excess of O group in duodenal ulcer and of A group in gastric carcinoma has been demonstrated. There was an indication, though not significant, for the excess of O group in gastric ulcer. The associations seem to be causal rather than due to population stratification, since the same tendencies have been observed in different populations. However, for the purpose of discussing the genetic consequences of selection resulting from these adult diseases, available data are still too scarce.

TABLE 8

JAPANESE DATA (TOKYO) FOR ASSOCIATIONS BETWEEN BLOOD GROUPS, PEPTIC ULCER, AND GASTRIC CARCINOMA

(After Uetake, Tanaka, and Fujii, [22])

PATIENTS	BLOOD GROUPS O	A	B	AB	PATIENTS VS. CONTROLS PROBABILITY O:A:B:AB	O:A
Peptic ulcer 695	260 (37.4%)	240 (34.5%)	141 (20.3%)	54 (7.8%)	$0.02>P>0.01$	$P<0.01$
Duodenal 163	71 (43.6%)	50 (30.7%)	29 (17.8%)	13 (8.0%)	$0.02>P>0.01$	$P<0.01$
Gastric 450	164 (36.4%)	163 (36.2%)	89 (19.8%)	34 (7.6%)	$0.20>P>0.10$	$0.20>P>.10$
Both 82	25 (30.5%)	27 (32.9%)	23 (28.1%)	7 (8.5%)	$0.70>P>0.50$	$0.80>P>0.70$
Gastric carcinoma 1565	439 (28.1%)	660 (42.2%)	318 (20.3%)	148 (9.5%)	$P<0.001$	$P<0.001$
Controls 1937	614 (31.7%)	722 (37.3%)	426 (22.0%)	175 (9.0%)		

For many years since the discovery of the blood groups by Landsteiner at the beginning of this century, it was generally believed that the blood group substances were of no particular significance in physiological and pathological processes. We have today many evidences that the blood group genes can no longer be regarded as neutral from the viewpoint of natural selection. The essential point of our present knowledge was predicted by Fisher (3) and was first proved to be true by Levine (11). Among persons of different blood groups there must be, to a greater or lesser extent, differences in resistance or susceptibility to diseases or in viability. Considering a population as a whole, the ABO polymorphism is undoubtedly due not only to random genetic drift but also to natural selection. In order to understand the dynamical aspect of this polymorphism in all its complexity, further studies must be carried out vigorously and on a much wider scale; the research will no doubt be of great significance both to human biology and medical science.

ACKNOWLEDGMENTS

The author is indebted to Drs. J. F. Crow at the University of Wisconsin and M. Kimura at the National Institute of Genetics in Mishima for reading the manuscript and for their helpful comments. Thanks are also due to Drs. M. Uetake, T. Tanaka, and K. Fujii at the Tokyo Medical and Dental College for permission to use their unpublished data.

REFERENCES

1. ALLISON, A. C. Aspects of Polymorphism in Man. Cold Spring Harbor Symp. Quant. Biol., **20**:239–55, 1955.
2. COHEN, B. H., and GLASS, B. The ABO Blood Groups and the Sex Ratio. Human Biol., **28**:20–42, 1956.
3. FISHER, R. A. The Genetical Theory of Natural Selection. Oxford: Clarendon Press, 1930.
4. GLASS, B. The Action of Selection on the Principal Rh Alleles. Am. J. Human Genetics, **2**:269–78, 1950.

5. GRUBB, R., and SJÖSTEDT, S. Blood Groups in Abortion and Sterility. Ann. Human Genetics, **19**:183–94, 1955.
6. INOUYE, E. Frequency of Multiple Birth in Three Cities of Japan. Am. J. Human Genetics, **9**:317–20, 1957.
7. ITOH, S. Selection by Incompatibility of Mother and Fetus with Respect to ABO Blood Groups, MN and Q Blood Types. Jap. J. Legal Med., **11**:128–49, 1957.
8. KIHARA, Y. The Problems of Blood Group Incompatibility in Obstetrics and Gynecology. J. Jap. Obst. Gyn. Soc., **6**:449–544, 1954.
9. LEVINE, P. Serological Factors as Possible Causes in Spontaneous Abortions. J. Hered., **34**:71–80, 1943.
10. ———. The Genetics of the Newer Human Blood Factors. Adv. Genetics, **6**:184–234, 1954.
11. LEVINE, P.; KATZIN, E. M.; and BURNHAM, L. Isoimmunization in Pregnancy, Its Possible Bearing on the Etiology of Erythroblastosis Foetalis. J.A.M.A., **116**:825–27, 1941.
12. MATSUNAGA, E. Intra-uterine Selection by the ABO Incompatibility of Mother and Fetus. Proc. Japan Acad., **29**:399–403, 1953.
13. ———. Reproductive Fitness of AB Children from AB × AB Mating. Sapporo M. J., **6**:165–68, 1954.
14. ———. Selektion durch Unverträglichkeit im ABO Blutgruppensystem zwischen Mutter und Fetus. Blut., **2**:188–98, 1956.
15. ———. Selection in ABO Blood Groups. Symposia on Twin Research and Population Genetics. Tokyo: Suppl., Jap. J. Human Genet., **2**:54–58, 1957.
16. MATSUNAGA, E., and ITOH, S. Intra-uterine Selection by the ABO Incompatibility of Mother and Fetus. (II). Proc. Japan Acad., **29**:529–31, 1953.
17. ———. Intra-uterine Selection by the ABO Incompatibility of Mother and Fetus. (III). *Ibid.*, **30**:677–82, 1954.
18. RACE, R. R., and SANGER, R. Blood Groups in Man. 2d ed. Oxford, 1954.
19. REED, E. T. Tests of Models Representing Selection in Mother-Child Data on ABO Blood Groups. Am. J. Human Genetics, **8**:257–68, 1956.
20. ROBERTS, J. A. F. Associations between Blood Groups and Disease. Acta genet., **6**:549–60, 1956.
21. STRANDSKOV, H. H. The Distribution of Human Genes. Sci. Mon., **52**:203–15, 1941.
22. UETAKE, M.; TANAKA, T., and FUJII, K. Relationship between Blood Groups and Diseases among Japanese with Special Reference to Gastric Cancer and Peptic Ulcer (to be published).
23. VAN LOGHEM, J. J., and SPAANDER, J. L'influence de l'incompatibilité du système ABO sur l'antagonisme Rh. Rev. d'Hém., **3**: 276–86, 1948.
24. WOOLF, B. ABO Incompatibility in Haemolytic Disease of the Newborn. Acta genet., **6**:519–20, 1956.

The Relation of the ABO and Rh Blood Groups to Differential Reproduction

BERNICE H. COHEN* and BENTLEY GLASS
The Johns Hopkins University, Baltimore, Maryland

In an earlier study (1), the authors have established the fact that in a Baltimore Rh-incompatibly mated series in which the mothers were nonsensitized (Rh N S series) there is a significantly low sex ratio (.48) in the A offspring of A mothers, whereas there is a significantly high sex ratio (.60) in the B offspring of B mothers. These results agree with those in the series of Rh unselected matings studied by Sanjhvi in Bombay and New York City, and by Johnstone in London. Because we felt the exclusion of the Rh-incompatible matings in which the mother was sensitized might present a bias, after it was learned that ABO-incompatibility offers protection against the occurrence of Rh sensitization, an Rh S series has been studied. In 2998 mother-offspring combinations, the same high sex ratio (.56) in the B offspring of B mothers was again found to occur; but no difference from a normal sex ratio was found in the A/A mother-offspring combinations (.53). The difference between the A/A sex ratios of the two series approaches statistical significance. Whether that difference is spurious or has meaning remains to be discovered. The explanation may, however, lie in the ABO interaction with Rh sensitization which has now been more extensively investigated in the Baltimore data. As Levine (2, 3) and others have shown, maternal-fetal incompatibility with respect to the ABO blood groups, such as occurs when the mother is of blood group O and the fetus of blood group A or B, exerts a protective effect against sensitization to Rh.

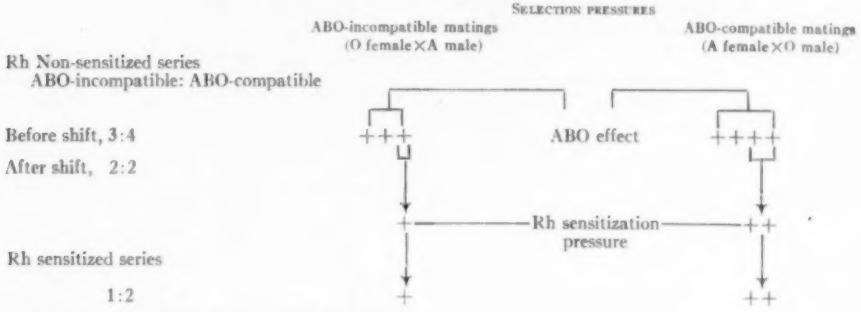
Extensive data from the files of the Baltimore Rh Typing Laboratory have been classified into two series, one in which the mothers are incompatibly mated with respect to Rh but are not sensitized (Rh N S series) and a second series in which the mothers, also incompatibly mated with respect to Rh, are sensitized (Rh S series). In these two series differential reproduction with respect to the ABO blood groups has been studied. Expectancies have been calculated from the general U.S. population, properly weighted for racial composition, as well as from the ABO frequencies found to exist in the parents belonging to the two series actually investigated. The Rh N S and Rh S series are very significantly different from each other, deviating in opposite directions from the expectation for the population. The Rh S series showed a significant deficiency of ABO-incompatible types and an excess of ABO-compatible types in mothers, fathers, offspring, and parent-offspring combinations. Conversely, the Rh N S series had an excess of ABO-incompatible types and a deficiency of ABO-compatible types. For example, in the Rh S series, among 2998 mother-offspring combinations, only 11.84 per cent were ABO-incompatible, as compared with an expected 20.94 per cent ($P < .001$); and among 1383 fertile matings only 25.96 per cent were ABO-incompatible, as compared with an expected 36.08 per cent ($P < .001$). In the Rh N S series, on the other hand,

* Public Health Service Research Fellow of the National Heart Institute.

among 3577 mother-offspring combinations, 22.92 per cent were ABO-incompatible, as compared with an expected 20.90 per cent ($.01 > P > .001$), and among 2313 fertile matings 38.82 per cent were ABO-incompatible, as compared with an expected 36.00 per cent ($.01 > P > .001$). (It should be noted that, if there were no interaction of ABO and Rh effects, the adverse effect of maternal-fetal ABO incompatibility would be expected to lower the frequency of ABO-incompatible offspring and matings in the Rh N S series, and not to increase them or leave them unaffected.) These deviations of the two series in opposite directions result from a preferential shift of ABO-compatible matings from the Rh N S group to the Rh S group, so as to leave a preponderance of ABO-incompatible matings in the Rh N S group (Table 1). This protection against Rh sensitization and hemolytic disease which is conferred by the presence of ABO-incompatibility not only affects the frequencies of Rh alleles, by tending to lessen the adverse selection against the *r* allele in populations in which this allele is less common than the various *R(D)* alleles; but, further, the advantage conferred by ABO-incompatibility may react also upon the ABO frequencies, so as to favor A and B in contrast to O. As a consequence of the ABO-Rh interaction, there is a resultant counteraction of opposing selection pressures: the selective disadvantage of ABO-incompatibles in the face of the primary selection pressure against A and B is counteracted by the selective advantage of ABO-incompatibles in the face of the negative Rh selection pressure (Table 2). Thus, within the Rh-incompatibly mated portion of the White population (13 per cent), ABO-incompatibility, instead of acting adversely to A and B, tends to increase their frequencies. Stated another way, double incompatibility at both ABO and Rh loci is superior reproductively to single incompatibility, i.e., Or ♀ × AR ♂ is superior to Ar ♀ × OR ♂. However, since the protection is not complete, the double incompatibility is not superior to, or even equal to, the lack of incompatibility seen in the reciprocal mating AR ♀ × Or ♂. Since the zygotes involved in the double incompatibility are the double heterozygotes, this phenomenon may be described as the superior-

TABLE 1

THE PREFERENTIAL SHIFT IN RH-INCOMPATIBLE MATINGS OF ABO-COMPATIBLE MATINGS IN COMPARISON WITH ABO-INCOMPATIBLE MATINGS



Each "+" represents an equal number of matings.

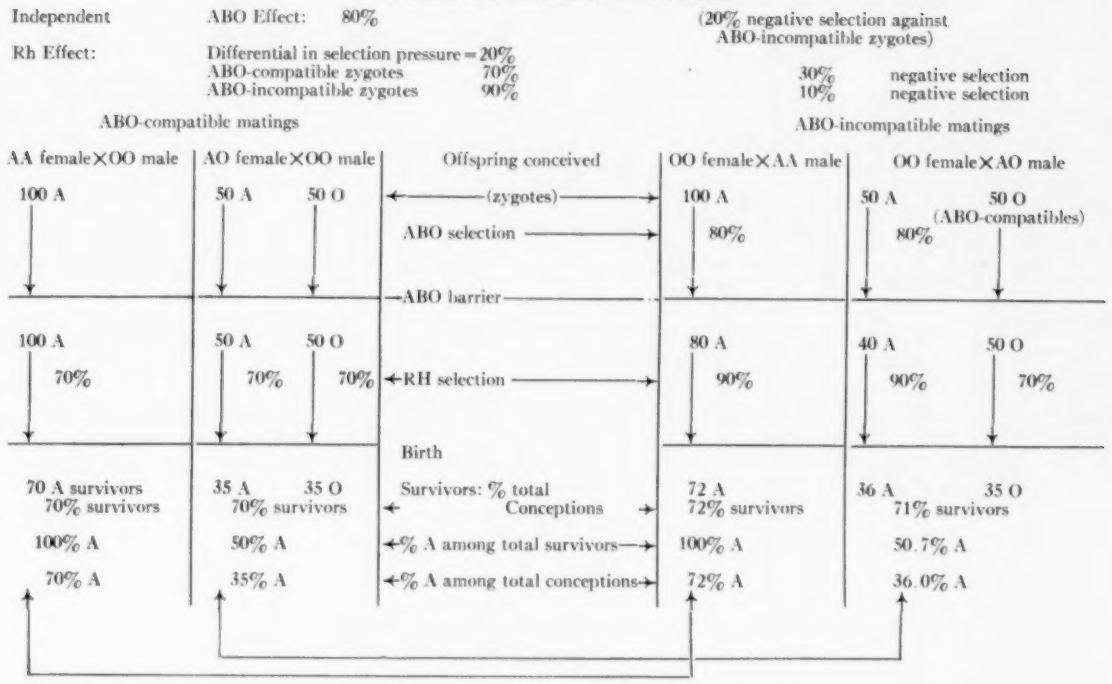
The primary ABO effect is represented by the reduction in number of +'s (fertile matings) representing the ABO-incompatible matings as compared with the ABO-compatible matings (i.e., 3:4).

The differential in Rh sensitization pressure is represented by the number of +'s shifted relative to the number belonging to the mating category before the shift (i.e., 1/3 for O × A matings; 2/4 for A × O matings).

TABLE 2

OPPOSING SELECTION PRESSURES IN RH-INCOMPATIBLE MATINGS

A SIMPLIFIED HYPOTHETICAL EXAMPLE



ity of the double heterozygotes over the single heterozygote. This superiority is, of course, restricted to the presence of the zygote in the incompatibly mated mother.

The protective action of ABO-incompatibility is estimated, from the Baltimore data, to fall between 5 and 15 per cent of the total Rh-incompatible matings, or most probably about 10 per cent. The protection would, therefore, extend to little more than 1 per cent (10 per cent of 13 per cent) of the total matings in the population. It is consequently somewhat unlikely that the protective effect could largely offset the negative selection against A and B owing to the ABO effect in the major, Rh-compatibly mated portion of the population. Nevertheless, the ABO-Rh interaction remains a factor in the complex of selective forces acting on the frequencies of the ABO alleles.

There is some evidence in the Baltimore data that the protection against Rh hemolytic disease which is conferred by ABO incompatibility is not limited to inhibiting or retarding Rh sensitization, but extends also to cases in which the mother has already become sensitized. This may be seen from the following: (*a*) there is a regular rise in the percentage of A offspring in successive pregnancies (O × A matings) within the Rh S series; (*b*) there is a higher pregnancy wastage in O × A (incompatible) matings of the Rh N S series than in A × O (compatible) matings, but a lower pregnancy wastage in O × A matings than in A × O matings of the Rh S series; (*c*) in both series, O × A matings have a significantly higher frequency of spontaneous abortions, attributable to the ABO effect, than do A × O matings; but there is a significantly *lower* frequency of stillbirths, attributable to the Rh effect, in the O × A matings than in the A × O matings of the Rh S series; and, finally (*d*) in both series O × A matings show more live births than A × O matings. If ABO incompatibility indeed confers further protection against Rh hemolytic disease after the sensitization of the mother, then the counteraction of the primary negative selection pressure on the A and B allele frequencies will be greater than was estimated above.

Regardless of the magnitudes of opposing selection pressures in the actual case, this interaction of ABO and Rh incompatibilities is significant in providing a model to illustrate the complexities of selective forces. In human blood group systems, the interaction of two negative selection pressures can, in fact, under certain conditions, yield a positive selection pressure, quite as Timoféeff-Ressovsky (4) long ago demonstrated might occur when certain deleterious mutants in Drosophila are combined.

REFERENCES

1. COHEN, B. H., and GLASS, B. The ABO Blood Groups and the Sex Ratio. Human Biol., **28**:20–42, 1956.
2. LEVINE, P. Serological Factors as Possible Causes in Spontaneous Abortions. J. Hered., **34**:71–80, 1943.
3. ———. The Influence of the ABO System on Rh Hemolytic Disease. Human Biol., **30**:14–28, 1958.
4. TIMOFÉEFF-RESSOVSKY, N. W. Über die Vitalitat einiger Genmutationen und ihrer Kombinationen bei *Drosophila funebris* und ihre Abhängigkeit vom "genotypischen" und vom äusseren Milieu. Ztschr. indukt. Abstamm.-u. Vererblehre, **66**:319–44, 1934.

The Protective Action of ABO Incompatibility on Rh Isoimmunization and Rh Hemolytic Disease—Theoretical and Clinical Implications*

PHILIP LEVINE, M.D.
Ortho Research Foundation, Raritan, N.J.

The combined series of eleven independent studies including 5,146 matings reveal that, in contrast to 35 per cent ABO incompatible matings in random matings, there is an average of 17.7 such matings in Rh hemolytic disease. In all ABO incompatible matings the husband is heterozygous for A or B. Husbands of genotypes AA or BB are excluded. In contrast to 59 matings, 0 Rh-positive husband by AB Rh-negative wife, there was only one reciprocal AB × O mating.

The essential feature for production of Rh antibodies is the survival in the maternal circulation of Rh-positive group compatible fetal red cells for long enough periods to reach the maternal sites of antibody production. If the fetal red cells are ABO incompatible with maternal anti-A or anti-B, they are immediately agglutinated and hemolyzed in the uterine sinuses so that the Rh antigen is not available to provide the antigenic stimulus. These findings provide indirect evidence that in a normal pregnancy the intact fetal red blood cell finds its way into the maternal circulation. More direct evidence is provided by Richardson-Jones who actually demonstrated fetal Rh-positive red cells in the blood of the Rh-negative mother.

The same principles apply also to hemolytic disease due to anti-c, provided that immunization by transfusion is excluded. Here again there are 18 per cent ABO incompatible matings instead of the random 35 per cent. Still additional evidence is provided by the results of immunization of Rh-negative volunteer donors with ABO compatible and incompatible Rh-positive blood (Stern *et al.*).

The chances for production of Rh antibodies are 2.4 times greater in ABO compatible matings than in ABO incompatible matings. (Calculations made with Prof. Howard Levene.)

If fetal red cells enter the maternal circulation, one may assume also a two-way transfer of leukocytes. This may perhaps explain findings of Peer of prolonged survival in 25 per cent of the cases of skin transplants from infant to mother and mother to infant, in contrast to uniform rejection of skin transplants from father to infant and infant to father.

* These findings have been published in the February, 1958, issue of Human Biology and in Memoir No. 86 of the American Anthropologist.

Discussion: Selective Factors in the ABO Polymorphism

J. A. BUCKWALTER, M.D.
Department of Surgery, College of Medicine, State University of Iowa, Iowa City, Iowa

In Chart 1, the associations of statistical significance which have emerged from investigation of selective factors manifest in human disease, in the population of the State of Iowa (Buckwalter *et al.* [2]) related to ABO polymorphism are shown. Data from prospective studies in progress for about a year in no instance have run counter to the findings of these retrospective ones. As a result, differences between patient and control ABO blood type frequencies become more, rather than less, significant.

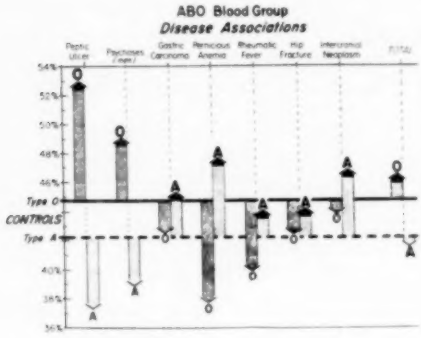


CHART 1.—ABO blood group disease associations

The data from studies of disorders in which equivocal or no statistically significant differences were found, patients from controls (voluntary blood donors), are shown in Chart 2. The findings for carcinoma of the lung and leukemia, although the differences are not statistically significant, are such as to suggest the collection of more data.

One explanation for the continued heterozygosity of the ABO blood groups is a dynamically balanced set of selective factors (Brues [1]), such as the disorders being discussed. Some support for this hypothesis is provided by comparing the composite (6936 patients) blood type frequency curve for the seven patient groups in which statistically significant disease control differences have been found with the composite (3962 patients) curve for the eight patient groups in

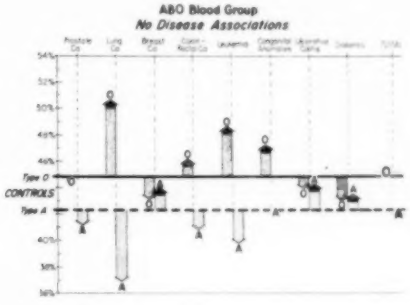


CHART 2.—No ABO blood group disease associations.

which there is no evidence for an association (Chart 3). The differences balance one another, causing the association curve to approach (*a*) the control curve and (*b*) the curve for those diseases in which no association was found.

Population stratification remains a pos-

sible explanation for the ABO blood group disease association. Note that the findings for gastric carcinoma and peptic ulcer in a four South African racial groups may provide a more conclusive answer to the stratification question.

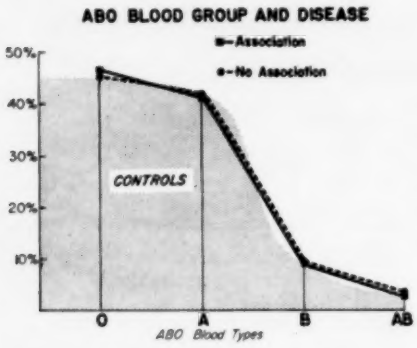


CHART 3.—Composite blood type frequency curves: patients, controls (voluntary blood donors).

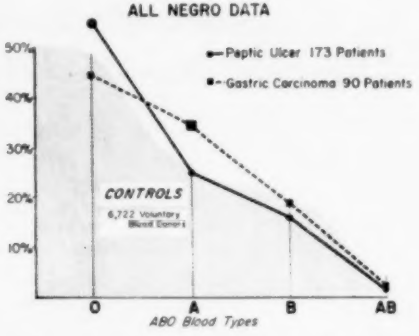


CHART 4.—Blood type frequencies in Negroes: gastric carcinoma and peptic ulcer patients, controls (voluntary blood donors).

Negro population (Buckwalter *et al.* [3]) are similar to those reported for Caucasian populations, i.e., increased incidence of gastric carcinoma in A and peptic ulcer in O type individuals (Chart 4). The Negro data, however, are not statistically significant. Also remember that this was not a pure ethnic group. It is anticipated that the findings of an investigation by the discussant of the ABO blood groups disease association in

REFERENCES

1. BRUES, A. M. Selected and Polymorphism in the ABO Blood Groups. Am. J. Phy. Anthrop., **12**:559–97, 1954.
2. BUCKWALTER, J. A.; TIDRICK, R. T.; KNOWLER, L. A.; WOHLWEND, E. B.; COLTER, D. C.; TURNER, J. H.; RATERMAN, L.; GAMBER, H. H.; ROLLER, G. J.; POLLOCK, C. B.; and NAIFEH, V. K. The Iowa Blood Type Disease Research Project. J. Iowa State M. Soc., **48**:70–81, 1958.

Comment

ERNST MAYR
Museum of Comparative Zoology, Harvard University, Cambridge, Mass.

In an endeavor to study disease-blood group correlations at the pre-reproductive age level, L. K. Diamond and I have recently analyzed over 5,000 admissions to the Children's Hospital at Boston.[1] When subdivided according to diagnosis, the largest subpopulation as a whole and can be used as control sample (Table 1). The ABO composition of admissions for various types of anemias tended to deviate from that of the control group (Table 1). Among "metabolic" anemias and related conditions

TABLE 1

BLOOD GROUP FREQUENCIES AND PERCENTAGES

	O		A		B		AB		
	N	Per cent	N	Per cent	N	Per cent	N	Per cent	TOTAL N
Children's Hospital, Boston:									
Congenital anemias	36	57	20	32	6	9	1	2	63
Metabolic anemias	65	33	92	47	30	15	10	5	197
Congenital heart diseases	583	45	532	41	140	11	48	4	1303
Boston population	55,089	45.8	47,752	39.7	12,990	10.8	4,450	3.7	120,281

χ^2 O against A + B + AB of metabolic anemias vs. congenital heart diseases = 9.63 (P = 0.02).

TABLE 2

BLOOD DISEASES ASCRIBED TO DISORDERS OF METABOLISM, GROWTH, OR NUTRITION

(Children's Hospital, Boston)

Name*	O	A	B	AB	Total
Hypochromic microcytic (iron deficiency) anemia	37	51	21	7	116
Pancytopenic anemia	6	10	3		19
Thrombocytopenic purpura	16	26	4	3	49
Others	6	5	2		13
Total:	65	92	30	10	197

* According to Plunkett, *Standard Nomenclature of Diseases.*

sample consisted of babies with various types of congenital heart defects (N = 1303). These heart cases, separated according to diagnosis or pooled, agreed closely in blood group composition with the Boston (N = 197) there is an excess of A and B (Table 2). Among "congenital" anemias (N = 63) there is a possibility of an excess of O; this includes aplastic, hypoplastic, and hereditary hemolytic anemias. We are continuing this work in an endeavor to increase the size of our samples and to determine the possible statistical significance more clearly.

[1] This investigation was supported by research grant H-2409 from the Heart Institute of the National Institutes of Health.

Discussion on Somatic Cell Genetics

GEORGE KLEIN
Department of Tumor Biology, Karolinska Institutet, Stockholm 60, Sweden

To study the occurrence and the nature of somatic variation under *in vivo* conditions, it is essential to have suitable and specific marker genes. It is also necessary to work out selective systems that enable the detection of specific gene products in single cells and their clonal derivatives.

The histocompatibility genes of the mouse seem to represent the only available system at present that can meet these requirements. By the use of Snell's isogenic resistant (IR) mouse lines, it is possible to restrict genetic variation to one small chromosome segment. For the study of somatic cells, F_1 hybrid mice seem to be particularly suitable, produced by a cross between two IR lines and differing only with regard to the allelic substitution at the H-2 (histocompatibility-2) locus. Allelic differences at this locus are known to constitute an exceptionally strong barrier against homotransplantation. Normal and neoplastic tissues of such F_1 hybrids are usually only transplantable to genetically identical hybrid hosts but not to any of the parental strains, where they provoke a homograft reaction directed against the isoantigens determined by the H-2-allele derived from the opposite parental strain. Mutation of one of the H-2 loci in a heterozygous tumor cell may convey a specific compatibility on its bearer, however, when tested in one of the parental strains, and would thereby become detectable. These considerations have led to the following experiments:

Sarcomas were induced by methylcholanthrene in four IR lines with an A/Sn genetic background and their various F_1 hybrid combinations. Model experiments involving the artificial mixture of known numbers of tumor cells derived from the parental strains and the F_1 hybrids have demonstrated that even a very small randomly admixed compatible cell fraction (with a frequency of about 4×10^{-7}) has an absolute selective advantage in its corresponding host in spite of the fact that the incompatible majority of the population is being destroyed completely by the homograft reaction, and even in cases where compatibility is a matter of a single gene difference. In fact, the homograft reaction directed against the incompatible majority stimulates the growth of the compatible minority.

A series of sarcomas was induced by the same dose of methylcholanthrene in the same tissue of various F_1 hybrid combinations of the four coisogenic resistant lines. The tumors were tested by transplantation to the parental strains and other known genotypes. Seven tumors grew exclusively in their original F_1 hybrid host genotype and did not give rise to variant sublines in any of the parental strains under the conditions of testing. Eight tumors gave rise to a certain proportion of variants capable of growth in *one* of the parental strains. In most cases, the variants were found to be specific for the parental strain of selection upon subsequent testing. No variants were obtained in the other parental strain with these tumors. For each F_1 hybrid genotype, one of the parental strains was clearly preferred to the other as the favored site of variant formation. Six tumors tested gave specific variants in both parental strains. A certain predilection for one of the parental strains, characteristic for the F_1-combina-

tion used, was clearly expressed even in this case, however. Two tumors were "non-specific" and grew in both parental strains and also in foreign genotypes without discrimination. It was not possible to select variant lines with selective compatibility from these strains.

Some tumors and their selected variants were tested by serological methods, involving both hemagglutination and cytotoxic tests, for the presence of certain isoantigens determined by the H-2 system. The findings were closely parallel to the transplantation tests and revealed a specific loss of detectable isoantigens concomitantly with the development of the ability to grow in one of the parental strains.

One tumor (MSWB) that regularly gave rise to a certain proportion of variants in one of the parental strains was examined in detail. Cytological evidence obtained by Bayreuther indicates that the occurrence of variants at different times represents mostly independent events. This was also confirmed by experiments involving the inoculation of very small cell numbers. As a rule, the variants differ both from the original line and from one another with regard to their chromosome cytology. Several lines have individually characteristic marker chromosomes.

In addition to the study of spontaneous variability in populations of tumor cells, histocompatibility genes seem to be excellent markers for investigations on possible recombination mechanisms. Studies of this type are under way.

The Study of Applicants, 1957–58

ERIC KLINGER* and HELEN HOFER GEE, PH.D.†
Association of American Medical Colleges, Evanston, Illinois

This study of the 1957 applicants to medical schools reveals that the trend toward a fairly rapid increase in the number of applicants that was predicted in the 1955 study, and seemed confirmed in the 1956 study, has been arrested. The predictions for medicine had assumed stability in the proportion of college graduates who would apply for entrance to medical schools and were based on predicted increases in the number of graduates. Inasmuch as figures on the accuracy of the undergraduate college predictions are not yet available, we do not know whether it is assumption, prediction, or both that have failed to materialize. From information that is available it appears likely that our assumption was wrong—that the proportion of college graduates applying for entrance to medical school has decreased.

TABLE 1

SUMMARY OF APPLICATION ACTIVITY DURING THE PAST 8 YEARS

Freshman year	No. applications	No. individuals	Applications per individual
1950–51	81,931	22,279	3.7
1951–52	70,678	19,920	3.5
1952–53	56,319	16,763	3.4
1953–54	48,586	14,678	3.3
1954–55	47,568	14,538	3.3
1955–56	54,161	14,937	3.6
1956–57	59,798	15,917	3.8
1957–58	60,951	15,791	3.9

The figures in Table 1 indicate that the trend toward an increase in the applicant population was not only arrested but reversed. Actually, however, the apparent decrease in number of applicants occurred entirely among repeat applicants (applicants who also applied for entrance in 1956–57). The size of this group decreased from 3168 in 1956 to 2995 in 1957.

Considering first-time applicants only, there was actually an increase of 47 students (see Table 10). However, such a small increase in a population of over 12,000 can hardly be viewed as consistent with the trend toward a large increase that was predicted.

Two other notable changes are revealed in the applicant population this year—changes in measured intellectual characteristics and changes in the pattern of application behavior. These are discussed in later sections of the report.

THE COMPETITION FOR APPLICANTS AND PLACES

Table 2 contains application information for each individual school. This table shows the number of freshman places filled, the number of applications received, the total number of applications filed by applicants (including applications to other schools), and the average number of such applications filed by each applicant. It is evident from Table 2 that the applicant situation faced by the different schools varies enormously. Although some schools receive but two applications for each place in their first-year class, other schools receive as many as eighteen. Large numbers of applicants to some schools apply to that school exclusively, but the typical applicant to other schools files ten or more applications.

* Research Associate, Association of American Medical Colleges.

† Director of Research, Association of American Medical Colleges.

TABLE 2

New Freshman Entrants and Application Activity of Applicants to Each School

School	No. new entrants in freshman class	No. applicants to each school			Total applications by each school's applicants	Av. no. applications made by each school's applicants
		Men	Women	Total		
Alabama	79	276	21	297	1,453	4.9
Albany	61	1,060	46	1,106	11,594	10.5
Arkansas	92	177	3	180	274	1.5
Baylor	84	913	30	943	6,819	7.2
Boston	73	1,119	92	1,211	11,833	9.8
Bowman Gray	54	772	40	812	6,643	8.2
Buffalo	79	769	32	801	8,095	10.1
California, L.A.	49	542	43	585	3,956	6.8
California, S.F.	79	384	27	411	2,837	6.9
Chicago Medical	72	1,059	31	1,090	11,357	10.4
Chicago, Univ. of	71	1,048	63	1,111	9,705	8.7
Cincinnati	91	1,024	27	1,051	8,409	8.0
Colorado	82	195	16	211	592	2.8
Columbia P & S	119	1,529	151	1,680	14,283	8.5
Cornell	84	1,209	116	1,325	11,750	8.9
Creighton	75	765	22	787	6,200	7.9
Dartmouth	24	318	2	320	3,091	9.7
Duke	79	819	46	865	7,079	8.2
Einstein (Yeshiva)	96	1,086	53	1,139	11,882	10.4
Emory	72	650	22	672	4,427	6.6
Florida	50	307	12	319	2,134	6.7
Georgetown	105	985	51	1,036	9,631	9.3
George Washington	101	1,313	57	1,370	12,736	9.3
Georgia	98	262	11	273	593	2.2
Hahnemann	108	1,108	44	1,152	9,299	8.1
Harvard	114	1,190	87	1,277	9,597	7.5
Howard	102	495	39	534	2,815	5.3
Illinois	190	511	38	549	2,422	4.4
Indiana	156	477	28	505	2,189	4.3
Iowa	118	198	5	203	429	2.1
Jefferson	175	1,748	1	1,749	12,887	7.4
Johns Hopkins	72	821	80	901	8,044	8.9
Kansas	101	348	12	360	1,918	5.3
Louisiana	117	282	16	298	811	2.7
Louisville	97	250	12	262	969	3.7
Loyola (Stritch)	88	796	33	829	5,813	7.0
Marquette	104	997	37	1,034	7,317	7.1
Maryland	94	328	10	338	2,601	7.7
Medical Evangelists	98	168	17	185	412	2.2
Meharry	69	417	30	447	2,010	4.5
Miami	78	214	13	227	1,001	4.4
Michigan	204	618	34	652	2,935	4.5
Minnesota	126	330	21	351	1,260	3.6
Mississippi	76	180	9	189	521	2.8
Missouri	73	184	6	190	570	3.0
Nebraska	84	228	11	239	776	3.2
New York Medical	127	1,692	102	1,794	16,889	9.4
New York University	132	1,242	93	1,335	13,464	10.1
North Carolina	66	267	17	284	1,443	5.1
North Dakota	40	96	4	100	367	3.7
Northwestern	129	1,380	59	1,439	11,224	7.8
Ohio State	149	539	28	567	2,324	4.1
Oklahoma	99	250	12	262	942	3.6
Oregon	77	462	18	480	3,006	6.3
Pennsylvania	126	1,545	79	1,624	13,667	8.4
Pittsburgh	101	531	26	557	4,417	7.9
Puerto Rico	53	98	25	123	352	2.9
Rochester	71	1,064	56	1,120	11,039	9.9
St. Louis	116	1,226	43	1,269	10,111	8.0
Seton Hall	80	998	44	1,042	9,954	9.6

TABLE 2—*Continued*

School	No. new entrants in freshman class	No. applicants to each school			Total applications by each school's applicants	Av. no. applications made by each school's applicants
		Men	Women	Total		
South Carolina	80	194	9	203	386	1.9
South Dakota	43	247	16	263	1,962	7.5
Southern California	65	610	37	647	4,845	7.5
Southwestern	100	474	30	504	1,485	2.9
Stanford	62	479	34	513	3,941	7.7
State Univ. of N.Y.	150	1,230	91	1,321	12,975	9.8
State Univ. of N.Y.-Syracuse	80	1,052	42	1,094	10,825	9.9
Temple	133	1,452	83	1,535	12,161	7.9
Tennessee	196	537	18	555	1,451	2.6
Texas	126	520	37	557	1,592	2.9
Tufts	114	795	45	840	7,392	8.8
Tulane	132	1,013	36	1,049	7,405	7.1
Utah	55	295	11	306	1,765	5.8
Vanderbilt	51	736	28	764	6,765	8.9
Vermont	50	351	22	373	2,910	7.8
Virginia, Med. College of	84	338	25	363	1,853	5.1
Virginia, Univ. of	76	649	44	693	5,574	8.0
Washington, St. Louis	86	1,475	61	1,536	12,374	8.1
Washington, Univ. of	74	452	19	471	2,832	6.0
Wayne State	75	386	22	408	1,433	3.5
Western Reserve	81	1,286	86	1,372	10,470	7.6
West Virginia	40	126	9	135	457	3.4
Wisconsin	90	182	12	194	662	3.4
Woman's Medical	51	0	199	199	1,107	5.6
Yale	79	918	76	994	8,689	8.7
All Schools:	7,852	57,656	3,295	60,951*	.....	3.9

* Note that this figure is the total number of applications made by 15,791 individuals.

TABLE 3

APPLICATION ACTIVITY IN TAX AND PRIVATELY SUPPORTED MEDICAL SCHOOLS

Item	Support of School	
	Tax	Private
No. applications	16,719	44,232
No. places in freshman classes	3,859	3,993
No. applications per freshman place	4.3	11.1

It has frequently been asserted that geographic restrictions on the applicant population of tax-supported schools seriously interfere with their competitive position in the market for able students. Whatever the source of the interference, the figures presented in Table 3 bear out the fact that tax-supported medical schools do indeed receive far fewer applications per place available. Of course, since great diversity exists both among tax-supported and among private schools, the generalizations reported here are not descriptive of all schools within each classification. Nevertheless, taken as a group, the private schools received nearly three times as many applications for each of their 1957–58 first-year places as the tax-supported schools.

Other figures not tabled in the present report show that, although there is a strong relationship between the number of applications per place received by schools and the average Medical College Admission Test (MCAT) score of their accepted applicants, MCAT differences do not follow tax-private patterns. Hence, the tendency for more applicants to apply to private schools cannot be attributed simply to differences in MCAT scores.

The competitive position of tax-supported schools is affected by the fact that the average applicant to a tax-supported school applies to only four medical schools altogether, while the average applicant to a private school applies to a total of eight medical schools. It appears then that, although tax-

supported schools must choose from among many fewer applicants than are available to private schools of comparable size, they are somewhat more likely to succeed in securing the applicants to whom they offer acceptances.

Although Table 1 suggests that the competition for places in medical school is perceived by the applicant as severe enough to warrant increased application activity, the competition is actually slightly less rugged than in the past. The total number of applicants decreased, but Table 4 shows that the number of accepted applicants increased to 8302 from the 8263 of the preceding year. In percentage terms, 53 per cent of the total number applying were accepted (an increase of 1 per cent). The total number of enrolled freshmen also rose slightly, by 28 students, to 7852 (Table 2). In all, 50 per cent of the 1957–58 applicants actually enrolled in medical schools, but a total of 450 accepted students rejected their opportunity after having been accepted.

It is interesting to note (Table 4) that, whereas the total number of 1957–58 applicants decreased by 126, the number of applicants filing fewer than three applications decreased by 328. Evidently, this decline in applicants occurred disproportionately among that group whose application effort is relatively less vigorous, but this was accompanied by an increase in the size of the group whose application activity was heavy.

The shift may merely reflect substitution of many applications in one year for repeated applications in successive years by an invariant population segment. On the other hand, the possibility must be recognized that this shift of pattern in application activity may be symptomatic of a gradual general change in the segment of the population from which the medical profession must select its future membership.

Table 4 classifies applicants by acceptance, sex, and number of applications filed. Also shown are the percentages of applicants accepted in relation to the number of schools to which they applied. The results for the 1957–58 applicant group closely resemble those of 1956–57. Again it is apparent that filing more than three applications does not appreciably increase the applicant's chances of admission. There is little appreciable difference in the acceptance rates of applicants filing between three and fourteen applications. However, applicants filing more than fourteen, like those filing

TABLE 4

RELATIONSHIP BETWEEN APPLICANTS' BEING ACCEPTED AND THE NUMBER OF APPLICATIONS THEY FILED

No. applications filed	Applicants receiving one or more acceptances			Applicants receiving no acceptances			Total applicants			Per cent of total applicants accepted
	Men	Women	Total	Men	Women	Total	Men	Women	Total	
1	2,153	133	2,286	2,895	240	3,135	5,048	373	5,421	42
2	1,089	68	1,157	1,075	89	1,164	2,164	157	2,321	50
3	1,069	67	1,136	772	46	818	1,841	113	1,954	58
4	822	57	879	517	34	551	1,339	91	1,430	61
5	669	47	716	402	29	431	1,071	76	1,147	62
6	466	32	498	292	24	316	758	56	814	61
7	320	19	339	239	14	253	559	33	592	57
8	284	16	300	175	10	185	459	26	485	62
9	211	10	221	108	3	111	319	13	332	67
10–14	547	33	580	334	12	346	881	45	926	63
15–19	129	3	132	111	1	112	240	4	244	54
20–24	39	1	40	42	1	43	81	2	83	48
25–29	10	1	11	18		18	28	1	29	38
30–Up	7		7	6		6	13		13	54
Total:	7,815	487	8,302	6,986	503	7,489	14,801	990	15,791	53

fewer than three, fared less well in gaining admission than the members of the middle group.

The figures in Table 4 can be misinterpreted if it is not recognized that the number of applications filed by an applicant is correlated with other personal and situational characteristics. Figures in Table 5, for instance, indicate that, as the number of applications rises, MCAT scores also rise, except among those who file more than fourteen applications. The over-fourteen applications group may be trying to compensate with large numbers of applications for self-acknowledged deficits in their intellectual or academic qualifications. The applicant filing only one or two applications, on the other hand, is probably aiming primarily for admission to a local tax-supported school. This applicant may feel that his best chance of obtaining a medical education lies in seeking admission to a local tax-supported school where competition for places may be less severe and where residence restrictions may operate in his favor.

Table 6 gives the distribution of MCAT score means for enrolled students and applicants of the 85 medical schools and gives some indication of the tremendous variation that exists among schools with respect to

TABLE 5

MCAT SCORES OF APPLICANTS CLASSIFIED BY THE NUMBER OF APPLICATIONS THEY FILED

No. applications filed	Mean MCAT scores of accepted applicants					Mean MCAT scores of rejected applicants				
	N	Verbal	Quantitative	Modern society	Science	N	Verbal	Quantitative	Modern society	Science
1	2,232	514	516	514	507	2,590	460	453	468	438
2	1,143	520	517	523	515	1,108	458	449	470	435
3	1,129	524	517	522	513	800	463	451	467	439
4	879	531	516	528	519	539	466	448	464	444
5	715	537	523	533	521	423	477	447	474	444
6	498	538	518	534	519	313	482	456	477	453
7	339	550	522	542	522	249	478	448	479	448
8	299	548	514	545	527	185	489	459	494	450
9	221	548	519	544	527	110	487	455	489	460
10–14	579	549	516	547	529	346	487	457	485	457
15–19	131	537	506	549	515	111	503	474	510	469
20–24	40	525	478	533	509	43	488	447	494	450
25–29	11	554	499	568	525	17	449	406	442	427
30–34	4	550	493	533	530	5	541	461	551	467
35–39	2	525	440	590	495	.....	...	...	...	...
41	.....	...	...	...	...	1	505	405	585	505
49	1	555	525	505	415	.....	...	...	...	...
Average for total group:		528	517	527	516		467	452	472	442

TABLE 6

DISTRIBUTION OF MCAT SCORE MEANS OF ENROLLED STUDENTS AND TOTAL APPLICANT GROUPS IN 85 U.S. MEDICAL SCHOOLS

Av. MCAT score intervals	No. schools with mean scores at each interval							
	Verbal		Quantitative		Modern Society		Science	
	Enrolled	Applicant	Enrolled	Applicant	Enrolled	Applicant	Enrolled	Applicant
600 and over	3	0	1	0	1	0	1	0
550–599	20	5	16	0	24	5	18	1
500–549	44	40	44	19	41	44	35	23
450–499	16	37	22	60	17	33	26	52
400–449	2	3	2	5	2	2	5	8
350–399	0	0	0	1	0	1	0	1
Number of schools	85	85	85	85	85	85	85	85
Median of school means	526	503	520	480	528	507	516	482

the intellectual capacities of their students. This variation in measured learning ability is only one example of the great variation that exists also in other characteristics of individual schools and their students.

REPEAT APPLICANTS

A substantial proportion of each year's applicant group consists of "repeaters," that is, applicants who are reapplying after having been rejected during the previous year. Of the 1957–58 applicant group, 19 per cent, or nearly a fifth, had applied and been turned down in 1956–57. Information about this group appears in Tables 7 and 8. Table 7 shows the extent of their success in gaining admission to medical school according to their sex and the number of applications filed, and Table 8 presents their MCAT performance. These tables may be compared with Tables 4 and 5, which present identical information for the entire applicant group.

TABLE 7

RELATIONSHIP BETWEEN REPEAT APPLICANTS' ACCEPTANCES AND THE NUMBER OF APPLICATIONS FILED

No. applications filed	Total repeat applicants			Rejected repeat applicants			Accepted repeat applicants			
	Men	Women	Total	Men	Women	Total	Men	Women	Total N	Per cent
1	1,025	77	1,102	736	55	791	289	22	311	28
2	445	28	473	306	16	322	139	12	151	32
3	296	19	315	181	9	190	115	10	125	40
4	216	13	229	125	7	132	91	6	97	42
5	181	5	186	106	1	107	75	4	79	42
6	137	10	147	83	5	88	54	5	59	40
7	101	7	108	56	5	61	45	2	47	44
8	77	7	84	35	5	40	42	2	44	52
9	61	1	62	29	..	29	32	1	33	53
10–14	197	6	203	80	1	81	117	5	122	60
15–19	56	1	57	28	1	29	28	..	28	49
20–24	19	1	20	8	..	8	11	1	12	60
25–29	7	..	7	3	..	3	4	..	4	57
30–Up	2	..	2	...	..	...	2	..	2	100
Total:	2,820	175	2,995	1,776	105	1,881	1,044	70	1,114	37

TABLE 8

MCAT SCORES OF REPEAT APPLICANTS CLASSIFIED BY THE NUMBER OF APPLICATIONS THEY FILED

No. applications filed	Mean MCAT scores of accepted repeat applicants				Mean MCAT scores of rejected repeat applicants			
	Verbal	Quantitative	Modern society	Science	Verbal	Quantitative	Modern society	Science
1	502	501	496	496	451	442	464	435
2	488	501	499	484	448	442	464	427
3	504	507	504	504	459	450	465	447
4	513	514	516	509	465	448	462	450
5	513	516	515	486	479	461	474	453
6	503	505	495	482	465	443	474	445
7	532	518	527	510	458	429	480	442
8	536	517	538	510	488	468	488	466
9	544	523	543	527	474	457	495	460
10–14	528	512	530	523	479	469	481	461
15–19	528	510	531	505	491	465	474	458
20–24	507	442	472	472	494	484	468	471
25–29	503	508	583	540	468	445	432	438
30–Up	530	445	530	550	...	...	...	...
Total:	510	507	510	499	458	446	467	440

Of the repeat applicants, 37 per cent received at least one acceptance, as compared with 56 per cent of the first-timers. The 37 per cent figure indicates that the repeat applicants encountered greater difficulty in gaining admission than they found during 1956, when 39 per cent were accepted, and in 1955 when the per cent accepted was 40. Comparison of Table 8 with Table 5 readily reveals that the repeat applicants achieved MCAT scores appreciably lower on the average than those of the applicant group as a whole. As in previous years, the accepted repeaters fell far below the MCAT average of the entire accepted group.

TABLE 9

MEAN MCAT SCORES OF ACCEPTED APPLICANTS DURING THE PAST 6 YEARS

FRESHMAN YEAR	MEAN MCAT SCORE				STUDENT N
	Verbal	Quantitative	Modern society	Science	
1952–53	522	526	519	525	7,778
1953–54	519	525	524	530	7,756
1954–55	517	521	530	533	7,878
1955–56	524	528	527	522	7,969
1956–57	525	525	526	519	8,263
1957–58	528	517	527	516	8,302

TABLE 10

MEAN MCAT SCORES OF TOTAL FIRST-TIME APPLICANT GROUPS DURING THE PAST 6 YEARS

ENTERING CLASS FOR WHICH APPLICANTS APPLIED	MEAN MCAT SCORES				STUDENT N
	Verbal	Quantitative	Modern society	Science	
1952–53	497	497	495	493	11,548
1953–54	497	500	505	503	11,269
1954–55	494	496	508	504	11,895
1955–56	503	502	507	494	12,117
1956–57	500	498	504	487	12,749
1957–58	504	490	505	485	12,796

CHANGING INTELLECTUAL CHARACTERISTICS

In 1952, 1953, and 1954, Quantitative Ability and Science MCAT scores of accepted applicants were higher than Verbal MCAT scores (Table 9). From 1955 to the present the trend has been reversed, and the reversal has been increasingly accentuated, with Verbal scores gradually rising and Quantitative Ability and Science scores gradually falling. Although there has been considerable annual fluctuation, the trend is clearly apparent in these figures. The trend is not attributable to changing selection policies of admissions committees; the change lies in the population of students who are presenting themselves as candidates for admission to medical schools.

Table 10, which presents the MCAT scores of all first-time applicants for each of the years 1952–57, clearly shows that a decline in quantitative aptitude and scientific skills as measured by the MCAT has occurred, accompanied by a rise in verbal aptitude. Thus, the figures in Table 9 on MCAT scores of accepted applicants reflect the changing nature of the applicant populations with which admissions committees have been confronted. This finding contraindicates a "sputnik" effect on the applicant population and shows that the shift in the characteristics of the college student segment interested in making a career of medicine has occurred gradually over a period of years.

What factors might underlie a change in

TABLE 11

ACCEPTANCE DATA ON APPLICANTS BY STATE

STATE	APPLICANTS RECEIVING ONE OR MORE ACCEPTANCES				APPLICANTS NOT ACCEPTED			TOTAL NO. APPLICANTS	TOTAL NO. APPLICATIONS
	Men	Women	Total	Per cent	Men	Women	Total		
Alabama	115	7	122	56	87	10	97	219	497
Arizona	34	3	37	58	25	2	27	64	269
Arkansas	106	3	109	58	78	1	79	188	276
California	450	31	481	46	520	39	559	1,040	5,203
Colorado	84	5	89	57	59	7	66	155	319
Connecticut	95	7	102	50	97	4	101	203	1,330
Delaware	17	1	18	62	11	..	11	29	154
D. of Columbia	52	7	59	43	71	6	77	136	484
Florida	177	10	187	55	146	9	155	342	1,104
Georgia	166	8	174	50	168	6	174	348	724
Idaho	23	2	25	46	29	...	29	54	201
Illinois	390	29	419	57	294	23	317	736	2,675
Indiana	190	8	198	53	165	11	176	374	901
Iowa	161	5	166	71	64	5	69	235	506
Kansas	114	3	117	59	75	5	80	197	478
Kentucky	113	3	116	51	106	4	110	226	435
Louisiana	143	8	151	54	123	7	131	282	467
Maine	13	3	16	50	15	1	16	32	170
Maryland	108	7	115	53	97	4	101	216	725
Massachusetts	206	20	226	50	202	21	223	449	2,721
Michigan	303	14	317	51	279	24	303	620	1,779
Minnesota	168	9	177	62	100	10	110	287	618
Mississippi	125	8	133	61	81	5	86	219	412
Missouri	136	5	141	53	115	8	123	264	726
Montana	28	1	29	49	28	2	30	59	204
Nebraska	101	3	104	58	72	3	75	179	282
Nevada	2	1	3	25	9	...	9	12	43
New Hampshire	21	1	22	52	18	2	20	42	142
New Jersey	309	15	324	50	300	21	321	645	3,780
New Mexico	17	2	19	58	14		14	33	118
New York	1,021	76	1,097	50	1,024	80	1,104	2,201	15,371
North Carolina	153	8	161	51	145	12	157	318	869
North Dakota	46	2	48	73	17	1	18	66	135
Ohio	409	24	433	53	363	18	381	814	3,021
Oklahoma	133	3	136	60	82	7	89	225	485
Oregon	82	4	86	55	66	3	69	155	421
Pennsylvania	556	38	594	53	499	27	526	1,120	4,553
Rhode Island	29	1	30	42	38	3	41	71	519
South Carolina	99	5	104	44	122	8	130	234	449
South Dakota	35	5	40	67	17	3	20	60	138
Tennessee	219	7	226	74	76	2	78	304	472
Texas	332	20	352	51	322	22	344	696	1,838
Utah	65	2	67	57	50	...	50	117	351
Vermont	21	4	25	63	13	2	15	40	78
Virginia	157	13	170	59	107	11	118	288	742
Washington	99	1	100	60	63	5	68	168	554
West Virginia	66	4	70	45	78	6	84	154	486
Wisconsin	153	17	170	65	86	5	91	261	704
Wyoming	15	1	16	47	17	1	18	34	169
Puerto Rico	58	9	67	47	58	18	76	143	390
U.S. Possessions	21	3	24	34	43	4	47	71	303
Canada	5		5	09	49	2	51	56	182
Foreign	69	9	78	32	143	21	164	242	849
Not Stated	5	2	7	10	60	1	61	68	129
Total:	7,815	487	8,302	53	6,986	503	7,489	15,791	60,951

the nature of the medical school applicant population? Several possibilities suggest themselves, although none of these can be immediately verified. First, of course, is the increased emphasis placed by medical schools and their recruiters upon a well-rounded background of liberal education as a basis upon which to build a medical career. It is possible that this has reduced the importance placed by prospective applicants upon quantitative and scientific premedical background, and it may have led some college students to apply to medical school who previously would have been discouraged from doing so.

A related factor making for change in the applicant population might be the development of the concept of comprehensive medicine, which has led to the introduction of social and behavioral sciences into the medical curriculum. This may have stimulated an interest in medicine among students with higher verbal learning abilities. On the other hand, because the social and behavioral sciences are less well developed as sciences than are the physical and biological sciences, both college faculty and students may be prone to the misapprehension that comprehensive medicine and a medical curriculum that includes the study of social and behavioral phenomena are somehow less demanding of scientific aptitude.

Events outside the medical profession may also have contributed to changing applicant characteristics. The increased recruitment of students into basic sciences, mathematics, and engineering, along with considerably enhanced income prospects in many of these fields, may have lured away prospective medical applicants with particularly strong quantitative and scientific interests and abilities. A final possibility is that veterans of the Korean conflict, who would have begun applying to medical schools during the past 3 years, possess somewhat different intellectual and personality characteristics than non-veterans. It must be stressed that these possibilities are unverified, but it must be stressed also that their consequences are of the utmost importance to medical education and the medical profession.

TABLE 12

RELATION BETWEEN THE TYPES OF MEDICAL SCHOOL IN A STATE AND THE PERCENTAGE OF APPLICANTS FROM THAT STATE ACCEPTED TO SOME MEDICAL SCHOOL*

Type of medical school in state	Distribution of states according to per cent of their applicants accepted						Total no. states	Median per cent accepted
	Less than 35%	35–45%	46–56%	57–62%	63–68%	69–74%		
Tax only	0	2	6	9	2	2	21	58
Tax and private	0	0	10	2	1	1	14	53
Private only	0	1	5	0	0	0	6	50
Neither	1	1	4	3	0	0	9	49
All states	1	4	25	14	3	3	50	..

* Numbers in the body of the table (except in extreme right-hand column) are numbers of states. "States" here includes the first 48 states, the District of Columbia, and Puerto Rico. Apologies are due Alaska, which could not be included in the present tabulation without considerable inconvenience.

GEOGRAPHIC FACTORS

Geographic distribution of the 1957–58 applicants closely followed the pattern found in the preceding year (Table 11). Three states (New York, Pennsylvania, and California), whose combined populations comprised 24 per cent of United States population in 1957, contributed 28 per cent of the 1957–58 applicants. These three states, combined with four additional ones (Ohio, Illinois, Texas, and New Jersey), are the home states of just 50 per cent of the 1957–58 applicant group. New York, with 2201 applicants, contributed nearly twice as many applicants as the next state.

The percentage of applicants accepted also varies considerably from state to state (Table 11), ranging from 25 per cent in

TABLE 13

MEDICAL SCHOOL APPLICANTS AND ACCEPTANCES PER STATE POPULATION, 1957–58

State	Population* (×1,000)	No. applicants	Applicants per 100,000 population	Per cent of total applicants	No. acceptances	Acceptances per 100,000 population	Per cent of total acceptances
Alabama	3,136	219	6.98	1.4	122	3.89	1.5
Arizona	1,053	64	6.08	0.4	37	3.51	0.4
Arkansas	1,757	188	10.70	1.2	109	6.20	1.3
California	13,551	1,040	7.67	6.6	481	3.55	5.8
Colorado	1,624	155	9.54	1.0	89	5.48	1.1
Connecticut	2,257	203	8.99	1.3	102	4.52	1.2
Delaware	426	29	6.81	0.2	18	4.23	0.2
Washington, D.C.	798	136	17.04	0.9	59	7.39	0.7
Florida	4,111	342	8.32	2.2	187	4.55	2.3
Georgia	3,691	348	9.43	2.2	174	4.71	2.1
Idaho	640	54	8.44	0.3	25	3.91	0.3
Illinois	9,647	736	7.63	4.7	419	4.34	5.0
Indiana	4,499	374	8.31	2.4	198	4.40	2.4
Iowa	2,781	235	8.45	1.5	166	5.97	2.0
Kansas	2,058	197	9.57	1.2	117	5.69	1.4
Kentucky	3,003	226	7.53	1.4	116	3.86	1.4
Louisiana	3,022	282	9.33	1.8	151	5.00	1.8
Maine	923	32	3.47	0.2	16	1.73	0.2
Maryland	2,830	216	7.63	1.4	115	4.06	1.4
Massachusetts	4,775	449	9.40	2.8	226	4.73	2.7
Michigan	7,691	620	8.06	3.9	317	4.12	3.8
Minnesota	3,312	287	8.67	1.8	177	5.34	2.1
Mississippi	2,150	219	10.19	1.4	133	6.19	1.6
Missouri	4,210	264	6.27	1.7	141	3.35	1.7
Montana	666	59	8.86	0.4	29	4.35	0.3
Nebraska	1,424	179	12.57	1.1	104	7.30	1.3
Nevada	253	12	4.74	0.1	3	1.19	0.0
New Hampshire	566	42	7.42	0.3	22	3.89	0.3
New Jersey	5,572	645	11.58	4.1	324	5.81	3.9
New Mexico	789	33	4.18	0.2	19	2.41	0.2
New York	16,098	2,201	13.67	13.9	1,097	6.81	13.2
North Carolina	4,388	318	7.25	2.0	161	3.67	1.9
North Dakota	644	66	10.25	0.4	48	7.45	0.6
Ohio	9,186	814	8.86	5.2	433	4.71	5.2
Oklahoma	2,221	225	10.13	1.4	136	6.12	1.6
Oregon	1,739	155	8.91	1.0	86	4.95	1.0
Pennsylvania	10,990	1,120	10.19	7.1	594	5.40	7.2
Rhode Island	828	71	8.57	0.4	30	3.62	0.4
South Carolina	2,311	234	10.13	1.5	104	4.50	1.3
South Dakota	687	60	8.73	0.4	40	5.82	0.5
Tennessee	3,423	304	8.88	1.9	226	6.60	2.7
Texas	8,989	696	7.74	4.4	352	3.92	4.2
Utah	836	117	14.00	0.7	67	8.01	0.8
Vermont	368	40	10.87	0.3	25	6.79	0.3
Virginia	3,664	288	7.86	1.8	170	4.64	2.0
Washington	2,653	168	6.33	1.1	100	3.77	1.2
West Virginia	1,962	154	7.85	1.0	70	3.57	0.8
Wisconsin	3,855	261	6.77	1.7	170	4.41	2.0
Wyoming	310	34	10.97	0.2	16	5.16	0.2
Alaska and U.S. Possessions and Territories	738	71	9.62	0.4	24	3.25	0.3
Puerto Rico	2,272	143	6.29	0.9	67	2.95	0.8
Foreign	.....	298	.....	1.9	83	....	1.0
Not Stated	.....	68	.....	0.4	7	....	0.1
Total	.....	15,791	.....	100.1	8,302	....	99.7

* These figures are taken from *Current Population Reports*, Series P-25, No. 189, published November 13, 1958, by the Bureau of the Census. They are revised estimates of civilian population on July 1, 1957.

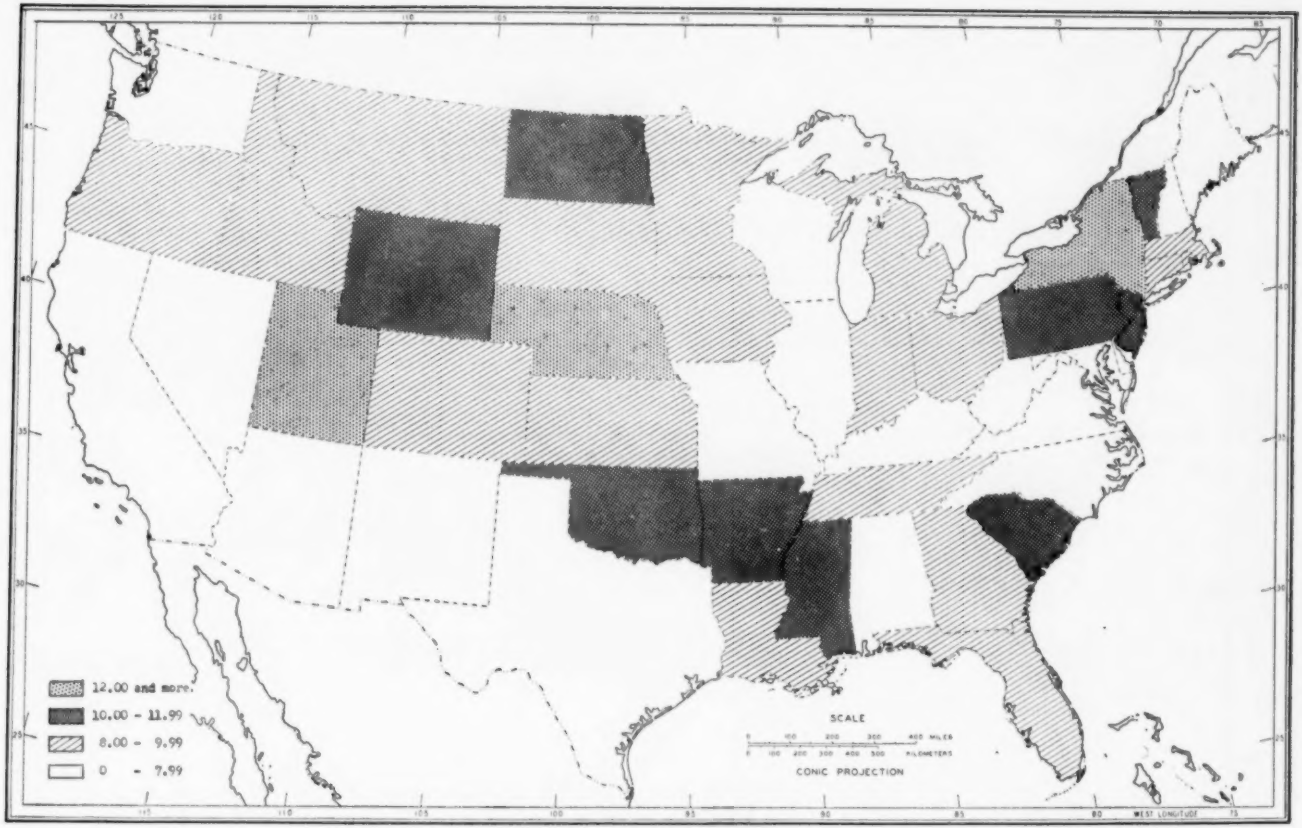


CHART 1.—Distribution of 1957–58 applicants per 100,000 population of their home states

Nevada to 74 per cent in Tennessee. The fact that these percentages are to some degree related to the availability of medical educational facilities is shown in Table 12, which tabulates states according to the percentage of their applicants who were accepted, and according to the types of medical schools that exist there. As in the preceding year, however, the statistical relationship is a weak one, and must not be construed as causal.

Table 13 provides some figures on the ratios of the number of applicants and accepted applicants to the populations of their home states. Again, great variation among states is evident, the number of applicants per 100,000 population ranging from 3.47 in Maine to 17.04 in the District of Columbia. In order to make the figures more meaningful, the numbers of applicants for each 100,000 in the population of their home states are shown pictorially in Chart 1.

Clearly, some groups of states provide much larger numbers of applicants in proportion to their population than do other groups. The Middle-Atlantic cluster, for instance, including New York, New Jersey, Pennsylvania, and Vermont, averages between ten and fourteen applicants per 100,000 residents, but most of the southern border and Pacific states from Louisiana to Canada average between four and eight. Just to the east of the latter belt, another group of states (Oklahoma, Arkansas, and Mississippi) contributes from ten to twelve applicants per 100,000. The variation among states does not follow East-West, North-South, or other such simple regional divisions, and no single factor could possibly account for existing differences. Contributing factors undoubtedly include the availability (or lack of it) of alternative desirable educational and occupational opportunities, the availability of medical educational opportunities, the degree of industrialization of the state, and the sociocultural heritage of the population. For example, ambitious young men of professional timber coming from essentially agricultural communities may find fewer attractive alternatives to a medical career than would their counterparts in metropolitan areas. On the other hand, medical education constitutes a high road to upward social mobility for the ambitious city dweller of recent immigrant stock.

Again, it is important to bear in mind that these reflections are unsupported speculations, advanced in lieu of specific evidence, but nevertheless concerning problems of significance to the medical profession.

CONCLUSION

This 1957–58 applicant study may be characterized by three distinguishing features: a possible decrease in the relative attractiveness of medicine among the nation's college graduates; a change in applicant behavior in terms of application activity, with fewer repeat applicants and larger numbers of applications; and an increasingly apparent shift in the intellectual qualities of the applicant population as measured by MCAT performance.

All three features are undoubtedly correlated to some degree, but only the latter gives cause for immediate concern. If only MCAT science achievement scores were declining among applicants, it would suggest that students were simply emphasizing breadth in their premedical preparation. The concomitant decline in quantitative learning ability scores, however, may indicate that medicine is attracting fewer students with the highest aptitude for scientific achievement. Insofar as clinical medicine can advance only as fast as the basic sciences on which it is based, this may reflect only a temporary shift in terms of the laws of supply and demand. But if the present shift continues to the point where the lowest echelon of accepted applicants becomes a group that is incapable of applying scientific advancement and methodology in the treatment of human illness, there will be cause for real alarm.

ACKNOWLEDGMENT

The authors are indebted to Patricia Bolan of the AAMC staff who processed the data and assembled the tables on which this study is based.

MEDICAL EDUCATION FORUM

Editorials

THE MEDICAL SCHOOL APPLICANT, A NATIONAL RESOURCE

At the outbreak of the Korean hostilities the Ford Foundation sponsored a survey related to manpower problems, and the ensuing report[1] underscored that, "The free world faces for an indefinite period a state of international distrust in which the numerical odds are greatly against it. Manpower and human skills will be the critical supply items for America for many years to come." The ensuing years have served to emphasize the truth of this assertion, and today we find that our intellectual stature has been equated to national survival. Under these terms of reference it should follow that the conservation of our human resources is a matter of national concern.

From the standpoint of manpower as related to medical education, one serious situation to which our attention should be drawn pertains to the applicant pool from which the schools of medicine select their matriculants, specifically to the problem of that selective restriction described as geographical discrimination. This is a distressing matter limited in great part to state-supported institutions and observed in its worst form where student allotments are assigned even down to the county level.

Demographers currently describe our American population as being in a fluid state, with evidence of a restless mass movement unequaled in the annals of our history. Under these circumstances it is somewhat naive to think in terms of containing education within state boundaries. Much more serious than the matter of simple provincialism is the fact that geographical limitation in the selection of matriculants excludes from our medical schools some of this nation's better minds. In the face of a chronic cold war in which the quality of our manpower is at a premium we can ill afford the petty luxury of the wastage of human resources.

While few will disagree with the valid assumption that the primary responsibility of a state institution lies in the education of its resident population, it is necessary for citizens of the state to appreciate the benefits of the healthy leavening of ideas and talents that accrue from the admission of well qualified non-resident students.

A recent study[2] of the physician population in Texas reveals that annually over the past 10 years one half of the total of approximately 600 licenses approved by the Texas State Board of Medical Examiners have been issued to physicians educated outside the State. There are three schools of medicine within the state of Texas, two of which are a part of The University of Texas system and the other being Baylor University College of

[1] A Report on the National Manpower Council, Graduate School of Business, Columbia University, 1954.

[2] Alfred G. Dale and Melvin A. Casberg, Illustrative Model Projection of Physician Population—A Preliminary Study in the State of Texas. The University of Texas Bureau of Business Research, 1957.

Medicine, a private school. The two former state institutions with a combined graduating class of some 230 students have been admitting only residents of the state to their freshman classes. Baylor, on the other hand, with a graduating class of approximately 80 students, admits one half of the freshman class from without the state. To complete this statistical picture, every year some 50 residents of Texas enter the freshman classes of medical schools elsewhere. Thus, over the recent years a total of some 320 residents of Texas have been graduating annually from all schools of medicine. However, this combined graduating class is only one half of the number of physicians annually taking up their practice in Texas. These statistics merely serve to emphasize how much a debtor a state may become to the medical schools of the rest of the nation.

Let us not forget that the medical school applicant is a national resource and as such should not be sacrificed on the provincial altars of geographical discrimination.

MELVIN A. CASBERG

FEDERAL SUPPORT FOR MEDICAL EDUCATION

The address of Dr. Lowell Coggeshall at the annual meeting of the A.A.M.C., in which he spoke out forcefully in favor of Federal support for medical education, focused attention on a critical problem. As one who has seen the problem, both as the Dean of a Medical School and as a ranking official in the Department of Health, Education, and Welfare, he was uniquely qualified to make an objective judgment on the question. In part, he reaffirmed an opinion officially expressed by the A.A.M.C. in 1957 favoring direct Federal support for medical education.

The necessity for an appropriate mechanism which would retain self control for the medical schools is, of course, essential. The program of the University Grant Committee through which the institutions of the United Kingdom and Northern Ireland receive direct governmental subsidy has been eminently successful and could serve as a pattern for us. The fact that these schools have been able to remain "free" with direct subsidy demonstrates that appropriate mechanisms can be established.

The year-to-year nature of the present Federal grants raises many problems. The University Grants program in Britain operates on 5-year allocations which encourage the kind of long-range planning so essential to sound educational and research developments. We believe that under the University Grants program the universities of the British Isles are freer than the medical schools of the United States. We are bound to a research enterprise which in truth controls many aspects of our activities.

For a medical school to be recognized as first-rate today, research accomplishment is at least as essential as educational accomplishment. Yet, who will deny that Federal funds for research have now outstripped the availability of medical scientists and the necessary research facilities? The medical schools cannot produce the scientists needed for an expanded research effort unless more adequate funds are available.

To those who oppose direct Federal support for medical education, these points should be emphasized. A very considerable percentage of the men who hold medical school appointments today receive their salaries through Federal grants-in-aid. Because they recognize that their security and research opportunity are dependent upon productivity, their

educational contribution is often minimal. In time, they may be more concerned about their stature in the eyes of the fund-granting agency than that of their fellow members of the faculty. The benefit to the general program of the medical schools would be far greater if these salary monies came in direct support of the educational program rather than through categorical research grants.

The fact that, at this time, the medical schools are unable to develop exact cost-accounting figures is no reason for deferring action. A variety of Federal programs operate on less firm financial projections and with far larger sums than would apply to a program for direct support of medical education. The study now being conducted by Mr. Augustus Carroll promises to produce the information that is needed.

The demand for more physicians cannot be met by the modest increases in funds that are available from endowments and through tax support at the State and municipal level. The medical schools have a big job now and a bigger one in the years immediately ahead.

Federal basic support for medical education seems the only answer. Such support should not be tied to projects or people, should be on a 5-year basis, and should be operated through an appropriate Committee of representatives of medical education.

JOHN Z. BOWERS

A HAPPY BIRTHDAY

The New York Times on January 22, 1959, paid tribute to the 75th birthday of Dr. Harry James Carman, Dean Emeritus of Columbia College. It was noted that one of his interests is "a number of educational surveys of great scope." On noting Dr. Carman's birthday, Joseph Hinsey commented on the contributions that he has made to medical education.

As a key participant in the Severinghaus study on Preparation for Medical Education, Dr. Carman brought to medical educators a fresh and invigorating approach to education. He demonstrated admirably the maxim that a great teacher is at home in any field. In the course of the Severinghaus study he visited many liberal arts colleges and medical schools. His sparkling report at the meeting of the A.A.M.C. in 1953 was memorable.

We wish him a belated but Happy Birthday, and we hope that he will continue to be concerned with medical education.

JOHN Z. BOWERS

Reports

THE DIRECTOR OF MEDICAL EDUCATION IN A HOSPITAL

The Director of Medical Education in a hospital should combine the most desirable characteristics of the chairman of a major clinical department with those of a dean. He should be certified by the "American Board of Diplomacy." In addition to clinical skill and enthusiasm for teaching, he should be able to stimulate and coordinate educational activities by other members of the Staff. His relations with hospital administration and the government board of the hospital should be cordial. These were a few of the points brought out in a Workshop Conference on The Role of the Director of Medical Education in the Hospital on January 16–17, 1959. Participants in the closed meeting included members of the Association of Hospital Directors of Medical Education, and representatives of the A.H.A., the A.M.A., and the A.A.M.C.

After a series of orienting talks, the first day was spent in group discussions which centered about questions concerning professional and administrative qualifications, role and responsibilities, clinical care, and relations with administration and staff.

The trend toward the appointment of the Director of Medical Education is a recent development, and the formal Association was established in 1956. The highly competitive situation relating to the procurement of interns contributed to the development of the position. However, throughout the Conference it was reiterated that the Director of Medical Education is an educator *not a recruiter*.

Although a variety of suggestions arose on how the Director of Medical Education should function, the reigning sentiment was to pick a good man and let him develop the job. In some instances, this might be a full-time job; in others, part-time; but, if a part-time job, the educational program of the hospital should be his prime responsibility.

The Director of Medical Education carries a joint responsibility both to the staff and, through the administration, to the governing board. The importance of staff cooperation and administrative support, including a budget, was emphasized. Staff support could be strengthened through the development of an active Advisory Committee.

In a masterful summarizing statement, Dr. Edward L. Turner pointed out that the importance of the educational aspects of intern and residency training is only now emerging in true perspective. Our concentration on undergraduate medical education has been predominant. Thus, the Director of Medical Education works in an area of transition where it is difficult to lay on specific parameters. It is important to recognize that this is a graduate area and teaching is primarily by precept. Not all hospitals need a Director of Medical Education, but, if the position is developed, the individual should be carefully chosen and supported.

A full report on the Conference will be available. Further discussions in this field will be of value in elucidating some of the complex problems of intern and resident training. Perhaps by increasing the educational role of the internship, the problem of an excessive number of programs which are solely service-oriented will approach a solution.

J. Z. B.

ABSTRACTS FROM THE WORLD OF MEDICAL EDUCATION

Analogue Computers for Research on Learning. W. K. TAYLOR. British Medical Journal, pp. 967–68. (Oct. 18), 1958 (London).

At the University College, London, theories of learning are being investigated with the aid of electronic analogue computing machines. These are based on the hypothesis —today widely accepted by neurophysicists —that learning is due to changes in interneuronal transmission characteristics. According to this theory, nerve impulses generated by the neurones which play a part in learning increase the transmission strengths of the paths they follow. Out of this hypothesis many problems arise concerning the physiological nature of the changes and the effects they would be expected to produce in different types of networks, and it is believed that construction of neural analogues helps to formulate and solve some of these problems.

Can Certification Be Separated from Graduate Education in Surgery? HERMAN E. PEARSE. Surgery, Gynecology & Obstetrics, Vol. 106, pp. 97–99 (Jan.), 1958.

In the field of surgery the rules for residency training set up by the Certifying Boards as a prerequisite to qualifying examination, were at first broad and liberal enough to permit the individual or institution to meet the requirements in a variety of ways. However, as the economic and professional rewards of board certification increased, rules were tightened more and more, and restrictive regulations—backed by the A.M.A. and the American College of Surgeons—have multiplied. There is now the tendency to "press everyone into the same mold." However, the basic issue in this matter is the invasion by regulatory bodies of the field of graduate surgical education, despite the fact that they are not educational institutions. Residency training, being an integral part of graduate surgical education, belongs, according to the author's view, under the jurisdiction of medical schools. The chairman of a Department of Surgery is best qualified to know the complex interrelationship between undergraduate and graduate education. He is the one who in the university clinic fosters, develops, and integrates all the branches of surgery, trying to coordinate the conflicting rules of the various specialty boards, and other regulatory bodies, with the over-all curriculum. Since the professor of surgery must take into account all aspects of graduate surgical education, he is also in the best position to decide on its needs. It should be possible, the author hopes, to separate examination and certification of competence from the educational process that leads up to it. In the present situation, when Boards specify the way to become competent, they encourage adherence to the letter rather than to the spirit of their rules. This has led many candidates to aspire to meet board requirements, rather than to acquire proficiency, and has caused many hospitals without educational facilities "to create half-baked residency programs which do not remotely resemble what the members of these boards had in mind." Furthermore, the boards of the various specialties of surgery have never co-ordinated their programs, and are not even in agreement on such a fundamental

issue as the determination of the amount of general training in surgery which should precede specialization. General training (often erroneously termed "general surgery") was considered in the past as a necessary part of any well organized residency program. Now the majority of surgical specialty boards require at least 3 years of specialized training, at the expense of a broad general training in surgery. Requirements as these, "pushed to the point of diminishing returns" militate against the full development of a special field. Although the prospect of reevaluation and reorganization of graduate education in surgery seems remote at this stage, the author believes that there is one—and only one—group able to do it. If the professors of surgery in the medical schools could agree—there is already a surprising unanimity of opinion among them—on what are the aims of specialization in the field of surgery and how to attain them, by their concerted action they could not only influence graduate education, but also end the Boards' attempts to rule education by edict.

Compréhension mutuelle entre le médecin specialiste et le médecin praticien (Mutual Understanding between the Specialist and the General Practitioner). GERARD CASGRAIN. *L'Union Médicale du Canada*, No. 10, pp. 1188–1193 (Oct.), 1958.

The present paper, read before the Canadian Academy of Allergy (meeting in Halifax June 24, 1958) by the Director of the Dept. of Allergy at the Hôtel-Dieu, Montreal, offers some reflections on the question of professional relations between general practitioner and specialist (this topic, it is pointed out, is seldom dealt with in medical literature). Speaking before an audience particularly interested in allergies, Dr. Casgrain's comments are based mainly on the experience of his own 16-year practice in this specialty. The gist of his observations, however, may as well apply to any other group of specialists as far as relationship with their colleagues in general practice is concerned. The author believes that this problem is essentially a question of professional competence and probity. At the basis of their co-operation—and co-operation is today, more than ever, a frequent necessity—there must be, above all, trust and confidence on the part of the referring doctor and essential probity on the part of the consulting doctor. This sharing of responsibility, Dr. Casgrain adds, is all to the benefit of the patient, and never has recourse to consultation harmed the reputation of the profession.

The Future Position of the Pathologist in Medicine. W. H. MCMENEMEY. The Lancet, pp. 841–44 (Oct. 18), 1958. (London).

Dr. McMenemey, pathologist at the Maida Vale Hospital for Nervous Diseases (London), offers a brief survey of the history of Pathology in England and examines its present position, as well as the influences at work today which are likely to modify the career of the pathologist in the future. In a hundred years from now, asks the author, will the pathologist be a scientist or a clinician, or a mixture of the two? Will future pathologists be doctors or technicians, or will they continue to be "precariously balanced"? Among the principal factors likely to affect the future career of a pathologist, the effects of the National Health Service on the profession are considered first. Under this system, pathologists, for many years to come, will be restricted in space, equipment, and personnel. The great load of diagnostic tests and scarcity of qualified technicians (underpaid by comparison with those in industry or in other governmental departments), and the overcrowded premises he works in, all combine to swamp the pathologist with routine investigations and prevent him from fulfilling his role as a trained observer and investigator, who could help bridge the daily widening gulf between hospitals and general practitioners. The recent advances both in diagnosis and treatment also greatly affect the pathologist's department: the increased use of blood, the plethora of antibiotics, radio-isotopes, im-

muno-hematology, etc., have increased demands on his laboratory time. Automation has already deprived the technician of some opportunity for exercising his skill, and rapid diagnostic procedures might soon be spreading in England the same as in the U.S.A. The universities also play a role in changing the aspects of pathology. Their task has been to foster research, especially in the experimental field. However, pathology is both a preclinical and clinical subject, and the principles of pathology are taught in university departments often detached from hospitals, whereas the practice of pathology is taught in hospital wards, laboratories, and the post-mortem room. There is now a school of thought that holds that chairs of pathology should be reserved for experimentalists and that hospital pathological appointments should remain within the Health Service. Such segregation, although it might permit more time for experimental research, would be unfortunate, thinks the author, because it would mean a cleavage between morbid anatomy and clinical pathology. Among British pathologists, especially the younger men, there is at present a wide-spread desire for the establishment of a College of Pathologists, which seems to be motivated principally by the need for higher examinations. That is, the need for the selection of high-level candidates. Since the inception of the NHS, the author thinks it is more than ever necessary that a pathologist, whatever his sub-specialty, should receive some training in all branches, and that special examinations should be instituted to test a candidate's ability for a career in pathology. The nature of these examinations should be determined by pathologists, and if the Royal College of Physicians is unable to provide for them, then an appropriate organization must be set up. The pathologist, says Dr. McMenemey, has evolved into the "consultant's consultant," and therefore a formula must be found which permits his influence to be diffused throughout all branches of the profession, in accordance with the demand, in our time, for integration in medicine.

Historique de la Neuro-Chirurgie (The History of Neurosurgery). JEAN SIROIS. Laval Médical, Vol. 26, no. 4, pp. 449–68 (Nov.) 1958 (Quebec).

That some sort of therapeutic trepanation was practiced during the Stone Age, and throughout the most ancient civilizations known, has been demonstrated without doubt by archaeological findings. The ancient Egyptians, Greeks, and Phoenicians practiced it in order to expel from the skull the "spirits" responsible for certain "crises" of the patients, or to repair damages caused by traumatisms. In the middle ages, Avicenna (980–1037) and some later physicians (such as Roger de Salerno in the XIIth, and Lafranc in the 13th century) describe in detail the traumatisms of the skull, indications for surgery, and its technique. Guy de Chauliac (1300–1368), the father of modern surgery, discusses the affectations of the nerves, pain, and certain methods of trepanation. Although the origin of our actual knowledge of the nervous system may legitimately be situated in the 14th century, five more centuries of tenacious work by men of exceptional insight were needed to penetrate into only part of the secrets of its structure, functions, and disorders, and it is only since the end of the 19th and beginnings of the 20th that the essential knowledge gained has permitted neurosurgery its real development. The second half of the 19th century was a great era for clinical neurology. Bouilland, Broca, Charcot, Jackson, and many other well known neurologists established during that period a great part of the pathology of the nervous system. Although the rapid evolution of clinical neurology during the last 30 years of the 19th century would indicate a parallel progress in the field of neurosurgery, this was not the case, because of one obstacle which seemed then invincible: infection, and the high mortality rate it caused. Without the work of Pasteur in France and Lister in England, neurosurgery could never have overcome so rapidly the technical difficulties it confronted. Among other pioneers in the field of neurosurgery whose contributions

are discussed in this study, Macewen (1848–1924), Horsley (1857–1916), and Cushing (1869–1939) should be mentioned. Progress in neurosurgery also owes much to Dandy (1886–1946), Frazier (1870–1936), Elsberg (1871–1948), Thierry de Martel (1870–1940), and many others. These pioneers, no matter how important the contribution of each, could never have expected, however, that they would help produce such an enormous development as neurosurgery has seen in the last few decades. At present, 37 countries have organized services in this specialty, and the great number of practicing neurosurgeons has brought about the foundation of seventeen societies and their World Federation (in Brussels, 1955). In the U.S. there are now 600 specialists in neurosurgery (by 1960 their number will be 800). Canada and all Central and South American countries possess neurosurgical services and societies, and so do Japan, Australia, and New Zealand. Although in Europe the growth of neurosurgical services has not been as spectacular as in America, England, France, and Sweden have produced eminent neurosurgeons and important innovations in methods and techniques which now are adopted universally. The last section of this study is devoted to a survey of new diagnostic techniques such as neuroradiology; electro-encephalography, and radio-isotopes. An extensive bibliography in neurosurgical literature (from Avicenna to the latest publications) can be found at the end.

Léonard de Vinci Pionnier de l'Anatomie Moderne (Leonardo da Vinci, Pioneer of Modern Anatomy). Robert M. Garraud. La Presse Médicale, pp. 1520–1522 (Oct. 1), 1958 (Paris).

Leonardo da Vinci applied the same passion to his scientific investigations that he devoted to his pictorial art. The present paper deals with his "cosmography of the minor world" (in a text of his *Quaderni d'Anatomia* he thus designs the human body), a treatise, or outline of a treatise, divided into 120 chapters, which is a transposition of Ptolemeian principles applied to anatomy. Leonardo had read Ptolemy's *Cosmography* (published in Rome, in 1490), and he states that in his description of the "minor world" he will follow the same method Ptolemy used in that of the universe, in accordance with his belief that man is a microcosm constructed in the same way as the cosmos and obeying the same laws. Leonardo's conception of the cosmography of the human body is outlined in this paper which emphasizes his most important contributions to modern anatomy, such as his discoveries in the domain of osteology, arthrology, and myology (the latter constitutes the greater part of his cosmography). Also studied in detail are Leonardo's concepts of the cardiovascular, digestive, and respiratory apparatus, and of the central nervous system, the complexity of which by no means discouraged him in his investigations (reproductions of the master's anatomical drawings illustrate the author's explanation). In his conclusions, Mr. Garraud points out that, although Leonardo must certainly be called an *amateur*, his genius appears in his anatomy studies, as in any other branch of science he investigated. It is to his great gift of observation that we owe, among other things, the first representations of the maxillary sinus, but of still greater importance are his didactic qualities, which, it can be said, opened the path to modern methods in anatomy. Although more perfect techniques have been developed in the course of time, the anatomist's thinking today still follows along the lines Leonardo da Vinci traced. And certainly nobody can deny him the title of being the genial creator of anatomical drawing. (For more detailed information on Leonardo's contributions to the science of anatomy see also two previous studies by the same author, in *La Presse Medicale*, 1957, no. 51, pp. 1218–1220; and 1958, no. 14, pp. 310–312).

Medicine's Challenge to the Educator. Sir George Pickering. British Medical Journal, pp. 1117–21 (Nov. 8), 1958 (London).

Sir George Pickering, Regius Professor of Medicine at Oxford, discusses in this paper (an address delivered to the Cardiovascular Program Directors of the U.S. Public Health Service, in Williamsburg, June 7, 1958) three issues which, as he confesses, are not only "three old hobby-horses" of his, but which deeply affect people's attitudes toward medicine and the teaching of medicine: (1) The issue of instruction versus education, (2) the importance of the proper use of words, and (3) the growing importance of population studies in clinical research. The principal point he raises in commenting on the first topic is the fundamental difference between *education*, that is, training of the mind, and *instruction*, "merely a process of forced feeding." The true aim of education is to impart an appreciation of method, rather than a knowledge of facts. Students must therefore be taught "how to collect the facts, verify them, to assign a value to them, and how to draw conclusions from them and test those conclusions; in short, how to form a judgment." In the U.S. and in Britain the "educational" approach has been made possible by a great increase in staff of the medical schools and a great development of postgraduate education. Two major obstacles, however, are seen in the rapid growth of knowledge and in the fact that there is almost no fact of natural knowledge that may not be turned to account in medicine. The difficulties consist in determining wisely the limits set to the fields of knowledge in which to practice the educational method, and in choosing the best methods of teaching. However, more important than the ground covered or the methods used is, in Dr. Pickering's view, the "attitude of mind" of the teacher himself. As to the second issue discussed, the correct use of language is absolutely necessary in order to transmit accurate information, and, the author says, it is for him a constant source of wonder "that we doctors are so little concerned with the accurate transmission of information within our own minds, and between other minds and ours." The chief cause of the prevailing misuse of language is seen in the use of technical jargon, "the way in which scientists fence off their subject with technical terms." To avoid a faulty transfer of information in matters so important as the taking of a case history, for instance, students should be taught to use the patient's own words, and never use terms such as "chesty," which means nothing, or technical terms such as "anginal pain," or "haemoptysis" that attempt to interpret the patient's experience. A consequence of the abuse of technical language is seen also in the danger of errors committed in the doctor's own mind. This point is illustrated by four examples, namely the terms "shock," "arteriosclerosis," "bronchospasm," and "hypertension." Lastly, the author discusses the importance recently being given, particularly in Britain, to the study of selected populations with the object of identifying the factors concerned in producing disease and the effects of treatment. In recent years, clinical science has been dominated by "phenomenology," that is, the application of the methods of physiology and biochemistry to the search for causal sequences in the individual patient. Although this method has doubtless proved its value, especially in demonstrating the precise action of drugs, the author thinks that much unnecessary effort is put into this form of investigation, which can determine nothing about the influence of genetic and environmental factors in the origin of disease. These factors can be assessed only by the scientific study of a population, which also has an important function in the appraisal of the effects of therapy. The method, developed in Britain by Bradford Hill (1951) under the name of "controlled therapeutic trial," is described at length and, as an example of its successful application, the British Medical Research Council's therapeutic trial of antituberculosis drugs is recalled (cf. also Dr. Marc Daniel's report, in *British Medical Bulletin*, 7:320, 1951). In the author's opinion, the *controlled clinical trial* represents the most important new method introduced into clinical science in recent years.

Milestones in Scientific Pediatrics. A. ASHLEY WEECH. A.M.A. Journal of Diseases of Children, pp. 553–58 (Nov.), 1958.

The truly great advances in medical science of the last few generations have been less concerned with the solution of specific problems than with the development of a new methodology which can be applied to expanding collateral fields. Some aspects of the conquest of rickets, which are discussed in detail in this paper (an address before the Am. Ped. Soc., Atlantic City, May 8, 1958), serve as an example of the fruitful collaboration between basic and medical science researchers which has grown out of this approach to medical science. Contributions from these sources are increasing constantly and, in the field of pediatrics, have been factors of utmost importance in its advances, especially concerning infectious diseases. Immunizing procedures, therapeutic sera, and antibacterial agents have vanquished some diseases completely (e.g., diphtheria and congenital syphilis), others have been "robbed of their horror." In the latter category, the author recalls the successful battle against all sorts of purulent meningitis and the prophylactic results in the campaign against rheumatic heart disease. But there are still conditions left which present a challenge for future conquests. It has been pointed out sometimes that pediatricians have so far contributed little in discovering antibiotics. Such statement can be considered true only in a purely literal way. In rebuttal, Dr. Weech stresses the fact that pediatricians were among the first to recognize the significance of certain events developing in this field. As an example, he cites the E. Mead Johnson Award of the American Academy of Pediatrics, given in 1941 to Rene Dubos, of the Rockefeller Foundation, who was neither a pediatrician nor even a M.D., because of the Academy's clear vision of the significance of his work: the study, over a decade, of naturally occurring enzymes capable of destroying organic compounds synthesized in living cells. By sheer inductive reasoning he had reached the absolute conviction that these enzymes simply had to occur in nature. Logic supported by experimentation culminated in the development of *gramicidin*. Another astonishing development, again not an original discovery but rather the effect of thinking in collateral fields, is the investigation reported by Linus Pauling and colleagues in 1949 (cf. Pauling, Itano, Singer and Wells, in *Science*, **110**:543, 1949) which deals with the identification by electrophoretic techniques of sickle hemoglobin and explains the nature of sickle-cell trait and disease on the basis of a single dose, as compared with a double dose, of an abnormal gene. The fact that Pauling is no pediatrician, says Dr. Weech, does not detract any value from this discovery. It should however be noted that Janet Watson, now teaching Pediatrics at the State University of New York, is also active in this field and that her explanation of the failure of sickle-cell disease to occur in very young infants has become a real milestone of progress. Another such mark of advance is seen in the comprehension of the causes of congenital malformations. The last decades have also seen great progress in techniques of corrective surgery and accurate diagnosis, most impressive in the management of congenital heart disease. In this connection, the author pays his greatest respect to Helen Taussig, not only for having visualized the benefits of an artificial ductus but also for demonstrating that accurate diagnosis is often possible on clinical grounds alone.

NEW BOOKS

Reviews

Patients, Physicians and Illness. Behavioral Science and Medicine. Edited by E. GARTLY JACO, with 64 contributors. Glencoe, Ill.: The Free Press, 1958. 592 pp. $7.50.

This book covers a wide range of topics, viewpoints, observations, and impressions; hence is appropriate for readers with quite diverse interests. Like many books, it is at times hard going in its early portions but becomes increasingly absorbing as progress is made through it.

The book is divided into seven major sections, namely: I. Social and Personal Components of Illness; II. Health and the Community; III. Socio-Cultural Aspects of Medical Care and Treatment; IV. The Patient: A Person with an Illness; V. Becoming a Physician: Medical Education; VI. Healing Practices and Practitioners; and VII. The Medical Setting: Hospital, Clinic and Office.

Each section is made up of a variable number of individual contributions, each an independent effect. Much of the material deals with presentation of research observations—mainly of a sociologic, economic, or social anthropologic —some well done and some highly questionable from the standpoint of well controlled data.

Portions of this book will be extremely interesting and valuable to medical educators in the sense of philosophy of medical education and the sociology of the medical student and the practitioner. In addition to focusing on the many different kinds of physicians in practice today, a brief look is taken at the osteopath and the chiropractor in the setting of today's society.

From the heterologous way this book is put together, it is possible—indeed advisable—to pick and choose sections to fit the reading time available. Every reader will doubtless find a wide range of interest in individual papers, and for those who like their reading smorgasbord there is much of interest here.

K. E. P.

Abstracts

Emergency War Surgery. Superintendent of Documents, U.S. Government Printing Office, Washington 25, D.C. 378 pp. $2.25.

This volume is the U.S. Armed Forces issue of the NATO Handbook prepared for use by the Medical Services of the NATO Nations. It is designed to cover the care of casualties in forward areas, from the battlefield to field and evacuation hospitals. The principles of practices described, however, are well adapted to the management of trauma anywhere. Each chapter ends with the practical application of the special principles and procedures described therein. A feature of the book is the detailed description of shock, together with a concise and precise presentation of the components and amounts of replacement therapy indicated in special circumstances. Another feature of the book is the very complete index.

Muir's Textbook of Pathology. By D. F. CAPPELL. 7th ed. London: Edward Arnold, Ltd. Distributed by Williams & Wilkins Co., Baltimore. 1171 pp. $14.50.

In this 7th edition, Dr. Cappell has retained the original objective of Sir Robert Muir in making this text for the medical undergraduate. Considerable rearrangement of the text has been carried out in the course of a thorough revision of many sections. The process of repair in special tissues, e.g., bone, nerve, has been brought into general chapter 3, the chapters on tumors have been extensively remodeled, chapter 7 is devoted primarily to consideration of the etiology of neoplasma, special attention has been paid to the rapidly advancing field of hematology, the section of fluid and electrolyte balance has been thoroughly revised, and the chapter on the endocrine glands has been virtually rewritten. About 100 additional illustrations have been incorporated, and many of the

older plates have been replaced by new and larger figures.

Viral and Rickettsial Infections of Man. Edited by THOMAS M. RIVERS and FRANK L. HORSFALL, JR. 3rd ed. Philadelphia: J. B. Lippincott Co., 1959. 924 pp., 134 illustrations. $8.50.

So much has been learned of viral and rickettsial infections during the 6 years since the second edition of this book was published, that it has been found necessary to completely rewrite the book. Thus the third edition is not a revision of the second edition, but rather a new book. Forty-four contributors to this book, fourteen more than participated in the preparation of the second edition, have all submitted newly prepared sections. The volume contains 46 chapters, seven more than the previous edition. This book is designed, as were the previous editions, to provide comprehensive information relative to viral and rickettsial infections which meets the needs of graduate students in biology, including those who are preparing for a career in medicine. It is hoped that it will also prove useful to physicians, teachers, and investigators of the biologic sciences.

Cardiac Arrest and Resuscitation. By HUGH E. STEPHENSON, JR., with 6 contributors. St. Louis: The C. V. Mosby Co., 1958. 318 pp. $12.00.

This book is devoted entirely to the problems of sudden cardiorespiratory failure and subsequent resuscitative procedures. The importance of an understanding of the features of cardiac arrest and resuscitation is unquestioned. But events have been moving so rapidly in this area in the past few years that it has been difficult for the average physician to keep abreast. The author of this book has established The Cardiac Arrest Registry to which physicians from all over the world have been contributing data. Many of the conclusions presented in this book are based on the study of over 1700 cases of cardiac arrest from this registry as well as from work done in the experimental laboratory of the author. A comprehensive bibliography has also been collected over the last 8 years. This book should prove valuable to almost every physician.

X-ray Diagnosis of the Alimentary Tract in Infants and Children. By EDWARD B. SINGLETON. Chicago: The Year Book Publishers, Inc., 1959. 342 pp. $11.00.

Many texts have been devoted to the roentgen study of the adult alimentary tract, but there is a scarcity of similar publications consigned solely to the pediatric patient. Because the technique of examination of the adult patient is not applicable to many pediatric patients, because the roentgen appearance of the digestive tract shows many variations in the pediatric age group not found in adulthood, and because many specific diseases of the pediatric digestive tract do not occur in the adult, this volume seems justified. It should prove valuable to radiologists and pediatricians alike in the early diagnosis of congenital and acquired alimentary tract abnormalities. The clinical aspects of the various abnormalities are briefly discussed in each section. Likewise, reference is made to the embryologic fault giving rise to the anomaly. Constant reference is made to the normal roentgen anatomy and attention called to the variations and differences in the normal occurring at different ages. In addition, the technique of examination of various portions of the digestive tract, especially in the infant age group, is included.

Management of the Newborn. By ARTHUR HAWLEY PARMELEE. 2d ed. Chicago: The Year Book Publishers, Inc., 1959. 351 pp. $8.50.

The first edition of this book, published in 1952, was well received. It is not meant to be a textbook in the ordinary sense but rather "a story of the newborn." The major portion of the book is devoted to the characteristics of the normal newborn. The present edition has had considerable revision, bringing into it considerable new knowledge. Retrolental fibroplasia, oxygen therapy of premature infants, and infections of the newborn as related to the staphylococcus are subjects which have been completely rewritten. Extensive revision has been made in the discussion of hyaline membranes syndrome, resuscitation of the newborn, and infants of diabetic mothers. Among the subjects that have been partially revised are congenital malformations, hemolytic disease, kernicterus, hemorrhagic disease of the newborn, and physiologic icterus. Many new references have been added to those appearing in the first edition.

Progress in Clinical Psychology, Vol. III. Edited by DANIEL BROWER and LAWRENCE E. ABT. New York: Grune & Stratton, Inc., 1958. 247 pp. $7.75.

This volume, like its predecessors, attempts a systematic and critical look at the contemporary scene in clinical psychology, particularly during the past 2 years. It attempts to integrate this material in a matter of interest and value to all—colleagues and students alike—whose concern is with clinical psychology as science. Many contributors have helped make this volume possible, presenting many specialized points of view. It is a prime object to point out not only what is going on in this active science, but what its defects are and what its promises for the future appear to be.

The Clinical Examination of the Nervous System. By G. H. MONRAD-KROHN. 11th ed. New York: Paul B. Hoeber, Inc., 1958. 453 pp. $9.50.

The revision of this book was with the significant help of Dr. Sigvald Refsum. In this edition, as in former editions, an effort has been made to strengthen the presentation of the purely clinical methods of observation without neglecting the technical auxiliary methods. Many components of the book have been significantly altered or expanded in accordance with newer knowledge, but, at the same time, an effort has been made to avoid any great enlargement of the book. As before, the book deals with the clinical examination of the nervous system and nothing more. It is not intended to be a textbook of nervous diseases, of which there are now so many. It remains a book written *from the clinic for the clinic*, based as much as possible on the personal experience of the author, and is not a compilation of all texts that have been advanced.

Practical Leads to Puzzling Diagnoses. Neuroses That Run through Families. By WALTER C. ALVAREZ. Philadelphia: J. B. Lippincott Co., 1958. 469 pp. $9.00.

The central theme to which this book is addressed has to do with the familial inheritance of nervous system disorders. The material for this book was gained by a thorough examination of the vast patient records of the author in which the family histories of the relatives of psychotic, alcoholic, and epileptic patients were abstracted to see what symptoms later showed up. In general, the author concludes that many of the relatives of the psychotic, alcoholic, and the epileptic are remarkably subject to queer symptoms and syndromes of various kinds—some of which appear now to represent minor equivalents of psychosis and epilepsy. In the strictest scientific sense all the material herein presented is not thoroughly documented. Rather, it is the objective of the author to stimulate the thinking of inquiring minds to look further into the subject for the purpose of proving or disproving some of the concepts presented. The one contribution the author is certain he is making is to stimulate in all physicians an awareness of the possibilities of inherited characteristics and traits such as to cause aberrant nervous system behavior.

Growth and Development of Children. By ERNEST H. WATSON and GEORGE H. LOWREY. 3d ed. Chicago: The Yearbook Publishers, Inc., 1958. 321 pp. $7.75.

In this edition the entire material of the previous edition has been re-examined in the light of recent progress. Most of that material has been retained, as it was thought to be useful and not out of date. However, changes and additions have been made in every chapter except the first one, which attempts to define some basic principles. The magnitude of these revisions may be appreciated from the fact that well over 100 new references have been added, which are reflected in numerous changes in the text. The chapter on the role of the endocrine glands in growth and development has been largely rewritten. The section on heredity has been extensively changed, and the chapter on behavior has had sufficient additions, especially concerning personality development, to modify its title. It is believed that in its present form the manual will prove useful to practitioners as a reference dealing with clinical problems in children and to workers in related fields, such as those concerned with public health and elementary education.

NEW THERAPY...TO DEFEAT THE MIGRAINE PARADOX*

'MIGRAL'®

TRADEMARK

- relieves headache
- dispels visual disturbances

and • overcomes nausea and vomiting

*The paradox of migraine – increased nausea due to ergotamine administration – may now be successfully combated with 'Migral'. The recognized benefits of ergotamine and caffeine in 'Migral' are favorably enhanced by the addition of cyclizine hydrochloride, a specific to overcome nausea.

Dosage: 2 to 3 tablets at first warning of an attack, then 1 or 2 tablets every half hour; not more than 6 tablets should be taken for any single attack.

Supplied: 'Migral' tablets, containing ergotamine tartrate 1 mg., 'Marezine'® brand Cyclizine Hydrochloride 25 mg., and caffeine 50 mg.

BURROUGHS WELLCOME & CO. (U.S.A.) INC., Tuckahoe, New York

NEWS FROM THE MEDICAL SCHOOLS

Alabama

Dr. SAMUEL C. LITTLE, formerly clinical professor of neurology, has been appointed professor of neurology and executive officer of the division of neurology at the Medical College.

Arkansas

Dr. DANA M. STREET, former chief of the Orthopedic Section of the Veterans Administration Hospital in Memphis, has been appointed professor and head of the orthopedic division of the surgery department.

Bowman Gray

At the recent annual meeting of the South Atlantic Association of Obstetricians and Gynecologists, Dr. C. HAMPTON MAUZY was installed as president. Dr. Mauzy is professor of obstetrics and gynecology at Bowman Gray School of Medicine.

University of Chicago

A special unit called the Section on Nuclear Medicine has been established in the School of Medicine to study the long-range impact of nuclear energy on matters of public health. The new unit, which will be constructed partly with a maximum of $500,000 by the Rockefeller Foundation and partly with funds supplied by the University and other sources, will deal with the full range of atomic public health problems from atmosphere to zoology. These problems will specifically include: the increase in natural background radiation due to by-products of all current uses of nuclear energy devices; the actual or probable consequences—genetic and physiologic—of the increased exposure of man and domestic animals to ionizing radiation; existing and proposed legislative controls of factors responsible for increased exposure to radiation; medicolegal aspects of personal injury and compensation problems of personnel engaged in the nuclear energy industry, and others; and the psychological reactions of society to the threatening aspects of nuclear energy. According to Dr. LOWELL T. COGGESHALL, dean of the Division of Biological Sciences, the work of the new Section will be of interest not only to his division but to other parts of the University or its affiliations, such as the Schools of Law and Business; the Argonne Cancer Research Hospital and the Argonne National Laboratory, both operated by the University for the U.S. Atomic Energy Commission; the Enrico Fermi Institute for Nuclear Studies; the Food Research Institutes; the National Opinion Research Center; and others.

Colorado

A third floor will be added to the structure which houses the Colorado Foundation for Research in Tuberculosis on the Medical Center campus. The addition, which will be constructed through a matching grant of $55,435 from the U.S. Public Health Service, will be used for the expansion of research in biochemistry and the genetics of bacteria. Dr. OSCAR REISS, presently with the Johns Hopkins University School of Medicine, will join the staff to direct the biochemical research activity.

Columbia

Effective July 1, Dr. ARTHUR GERARD DEVOE will become professor of ophthalmology and chairman of the department, as well as attending ophthalmologist and director of the Ophthalmology Service in Presbyterian

Hospital. Dr. DeVoe, who is now professor and chairman of the department of ophthalmology in the New York University Postgraduate Medical School, will succeed the retiring Dr. John H. Dunnington.

Dr. LEONARD J. GOLDWATER, professor of occupational medicine at the School of Public Health and Administrative Medicine, was recently named the recipient of a national citation from the President's Committee on Employment of the Physically Handicapped and the New York State Governor's Committee on "Employ the Physically Handicapped."

The School of Dental and Oral Surgery, which has been a unit of the Faculty of Medicine since 1945, will have independent status as the Faculty of Dentistry beginning July 1, 1959. Following a recent change made by the Columbia Trustees in the University Statutes, the School of Dental and Oral Surgery will have its own dean, Dr. GILBERT P. SMITH, who has been associate dean of the Faculty of Medicine for Dental and Oral Surgery for the past two years.

Georgetown

Dr. ROBERT F. LOEB, professor of medicine at Columbia University since 1938, will be awarded the Kober Medal for 1959. Established at Georgetown in 1923 by the Kober Foundation, the medal is being given in recognition of Dr. Loeb's contributions to the progress of the medical sciences.

The 1959 Kober Lecture recently was delivered by Dr. CHARLES A. HUFNAGEL, professor of surgery and director of the Experimental Surgery Laboratory at the School of Medicine. Dr. Hufnagel, who in 1952 performed the first successful substitution of a plastic heart valve for a diseased valve in a human patient, spoke on "The Basic Concepts of Cardiac and Vascular Reconstruction."

Hahnemann

Dr. JOHN J. SPITZER, associate professor of physiology, will head the new Laboratory for the Study of Lipids and Atherosclerosis, constructed with a $55,000 grant from the National Heart Institute. Other recently inaugurated facilities in the institution's planned expansion are the Institute for Biochemical Studies in Cancer and the Cytology Laboratory.

Illinois

Dr. PETER C. KRONFELD, who has been professor of ophthalmology at the College of Medicine since 1947, was recently appointed head of the department. Dr. Kronfeld, who succeeds Dr. WILLIAM F. HUGHES, also was named ophthalmologist-in-chief of the University Hospital and the Illinois Eye and Ear Infirmary. Dr. Hughes continues on the College of Medicine staff as clinical professor of ophthalmology.

Johns Hopkins

Dr. PALMER H. FUTCHER, associate professor of medicine, has been appointed assistant dean.

Professor ANDRE LWOFF, of the Pasteur Institute in Paris, delivered a Squibb Centennial Lecture at the School of Medicine recently on the subject of "The Evolution of Viral Disease at the Cellular Level and in the Organism."

Dr. ERNEST L. STEBBINS, dean of the School of Hygiene and Public Health, has been re-elected president of the Advisory Board for the Medical Specialties of the American Medical Association.

Kansas

Dr. CHARLES M. POSER, assistant professor of neurology and head of the section of experimental neurology, has been granted a one-year leave of absence to assume the position of executive medical officer of the World Federation of Neurology. This organization, which is located in Antwerp, Belgium, has been awarded grants totaling $138,207 by the National Institute of Neurological Diseases and Blindness. Dr. Poser will establish a central clearing house and information center for international neurological research.

Announcing a new text . . .

Robinson & Robinson: CLINICAL DERMATOLOGY FOR STUDENTS AND PRACTITIONERS

"Morphologic Dermatology was taught effectively at the University of Maryland School of Medicine for many years under the supervision of Dr. Harry M. Robinson, Sr. The present members of the staff of the Division of Dermatology have collaborated in the writing of this text in order to carry on this tradition.

"During World War II there was a rebirth of interest in dermatology. It became apparent to those interested in this field that a dermatologist must not be solely dependent on a casual visual examination but must study the entire patient in order to arrive at a definite diagnosis. The diagnostician should not be content with the clinical impression afforded by morphologic skin changes, but, through experience gained by years of study and practice, must correlate laboratory findings and clinical symptoms.

"The skin is not only a protective covering but is a functioning organ which has nervous, vascular, and hormonal communications with the viscera, central nervous system and the vascular system. It is the largest organ in the human body and frequently reflects disturbances in normal physiological functions, or pathologic changes elsewhere in the body.

"The art of diagnosis applied to dermatology requires careful inspection of the patient by the examining physician and a detailed record of abnormal findings. The major portion of the book is devoted to a discussion of the morphologic appearances of the more common dermatoses, methods of diagnosis, the relationship of cutaneous lesions to systemic diseases, and suggestions for treatment. Brief mention will be made of the more uncommon dermatoses."—*Preface*

By HARRY M. ROBINSON, JR., M.D., *Professor of Dermatology;* RAYMOND C. V. ROBINSON, M.D., *Assistant Professor of Dermatology; and the staff of the Division of Dermatology, University of Maryland Medical School*

Ready May • Approx. 300 pp., 117 figs. • Probable price: **$8.00**

Baltimore 2, Maryland

Miami

Creation of a new advisory Council of civic leaders for the School of Medicine was recently authorized by the Trustees of the University. The new Council will consist of twenty-one persons, of whom approximately one-fourth will be physicians. Its responsibilities will include the institutional and financial development of the medical school and all phases of the school's operation touching on the public interest.

Michigan

The National Institutes of Health has approved a grant of $322,000 to the College of Pharmacy for construction of a pharmacy research building on the Ann Arbor campus. A four-story structure, attached to a wing of the East Medical building, the greatest portion of the new building will be devoted to research laboratories accommodating two to four investigators, chiefly graduate students.

The American Academy of Pediatrics recently sponsored the first postgraduate course on pediatrics at the Medical Center.

Dr. H. MARVIN POLLARD, professor of internal medicine, and Dr. MAURICE H. SEEVERS, chairman of the department of pharmacology, will leave shortly to participate in a series of medical conferences in the Far East.

New York Medical

The third annual Van Slyke Award of the New York Section, American Association of Clinical Chemists, recently was presented to Dr. ISRAEL S. KLEINER, director of the department of biochemistry.

Dr. JAMES FREDERIC DANIELLI, professor of zoology at King's College, London, delivered one of the Squibb Centennial Lectures recently at the College, discussing his new theory of attacking cancer.

New York University

A grant of $300,000 has been received from the Fannie E. Rippel Foundation of Newark, New Jersey, toward the cost of the new University Hospital in the New York University-Bellevue Medical Center. This contribution is designated for the area in the hospital to be devoted to clinical research laboratories for the study of cardiovascular diseases, malignancies and degenerative processes of the aging. With the construction of the hospital, the Medical Center will have completed its building and development program begun in 1948.

Pittsburgh

Effective July 1, Dr. RICHARD J. CROSS, assistant dean of the College of Physicians and Surgeons, Columbia University, will become associate dean of the School of Medicine and assistant professor in the department of medicine. His chief responsibility will be the Medical School's postgraduate program.

Rochester

Dr. WALLACE O. FENN, chairman of the department of physiology and the last of the original department heads of the school, will retire June 30. He will continue as professor of physiology and associate dean for graduate studies at the School of Medicine.

Southern California

Reorganization of the administrative structure of the School has resulted in the following recent appointments: Dr. PETER V. LEE, associate dean for medical education; Dr. PHIL R. MANNING, associate dean for postgraduate education; and Dr. WILLIAM E. NERLICH, assistant dean for student affairs. Mr. ROBERT J. THOMAS, director of the Los Angeles County General Hospital, will represent the School of Medicine on the Committee of Hospital Administration of the AAMC.

Departmental changes include the promotion of Dr. THOMAS H. BREM to the chairmanship of the department of medicine, following the retirement to emeritus status of Dr. PAUL STARR.

Stanford

Dr. WINDSOR C. CUTTING, professor of experimental therapeutics, has been named a Fellow of the New York Academy of Sciences.

Named to succeed Dr. ROBERT H. ALWAY as head of the department of pediatrics is Dr. NORMAN KRETCHMER, currently associate professor of pediatrics at Cornell.

Tennessee

Dr. DAVID S. CARROLL, professor of radiology, was recently appointed head of the department to succeed Dr. CHARLES HUNTER HEACOCK, recently resigned, who will continue as professor of radiology.

A former associate professor of neurology at the State University of Iowa, Dr. ROBERT A. UTTERBACK is joining the staff as professor of neurology and head of the department.

University of Washington

Dr. H. STANLEY BENNETT, professor of anatomy and executive officer of the department, has been selected to give the summary lecture in the William P. Philips lecture series at Haverford College, Pennsylvania.

New appointed chief of the Microbiology Laboratory of the Teaching and Research Hospital is Dr. JOHN C. SHERRIS, a member of the University of Manchester faculty, England.

Dr. JOHN PHILIP SANDBLOM, president of the University of Lund, Sweden, has been appointed a Walker-Ames Professor of Surgery for a period of six weeks during March and April, 1960. While at the University, Dr. Sandblom, who is an honorary fellow of the American College of Surgeons and immediate past president of the Swedish Surgical Society, will lecture to medical students and residents, as well as present research seminars and demonstration operations.

West Virginia

The newly appointed coordinator of Medical Center activities is Dr. KENNETH E. PENROD, professor of physiology and assistant dean of the Duke School of Medicine, and book review editor for the Journal of Medical Education. Effective July 1, Dr. Penrod's duties will involve the internal coordination of all Medical Center programs, as well as assistance in the recruiting of faculty and staff personnel for the entire Center. The new $30 million plant, which, under construction since 1953, is scheduled to go into full function next year, is situated on its own 145-acre campus about a mile from the main University. It brings to West Virginia the state's first four-year medical training curriculum and the first dental school, and it coordinates these programs with the training of pharmacists and nurses and of technical specialists in the professional health field.

Easy to read at any distance

Tycos® HAND MODEL ANEROID

The specially designed gage is not attached to the cuff. With ample tubing it permits easy reading at any distance—even if you are near or farsighted. This is particularly convenient when examining very sick patients in bed.

Note these other important features:

1. Inflating bulb and air release valve built right into the back of the gage.

2. Balanced to fit comfortably in either hand.

3. New feather-touch valve control. Floating stainless steel ball replaces former needle valve for easier operation.

4. Single tube with Luer lock fitting—you can apply the cuff on your patient's arm before attaching the gage.

5. Accurate in any position—as long as pointer returns to zero.

Made by skilled American instrument technicians, the TYCOS Hand Model Aneroid carries the famous TYCOS 10-year triple warranty. Weighs only 18 ounces. With genuine leather zipper case and hook cuff, only **$49.50**, at your surgical supply dealer. Taylor Instrument Companies, Rochester, New York and Toronto, Ontario.

Taylor Instruments **MEAN ACCURACY FIRST**

ITEMS OF CURRENT INTEREST

Reserve Physicians may earn retirement points

Retirement point credits may be earned by reserve Medical Corps officers who attend the scientific sessions of the thirtieth annual meeting of the AERO MEDICAL ASSOCIATION in Los Angeles, California, April 27–29, 1959, according to an announcement by the Department of Defense. This authorization covers eligible physicians who are Medical Corps officers of the reserve components of the U.S. Army, Navy and Air Force on inactive status. Latest advances and developments in aerospace medicine of nearly 200 scientists from the United States and several foreign countries will be presented at the meeting.

American Heart Association Merger

The American Society for the Study of Arteriosclerosis has merged with the American Heart Association, the Society becoming the Council on Arteriosclerosis of the American Heart Association. This brings to eight the number of Councils now active in the Heart Association's program. The other councils cover such areas as clinical cardiology, cardiovascular surgery, circulation, high blood pressure, rheumatic fever and congenital heart disease, basic science, and community service and education.

American Hospital Association

Dr. EDWIN L. CROSBY, director of the American Hospital Association, recently announced the appointment of Richard L. Johnson of Des Plaines, Illinois, as an assistant director. Mr. Johnson, who will continue as secretary of the Association's Council on Administrative Practice, also was appointed director of administrative services. Dr. Madison B. Brown of Chicago, associate director of the AHA since 1957, was appointed director of professional services and secretary of the Council on Professional Practice.

Canadian Accreditation Program

On January 1, 1959, the Canadian Council on Hospital Accreditation assumed the responsibility of accrediting hospitals in Canada. To give its full support to this program, the Canadian Medical Association resigned its membership in the Corporation of the Joint Commission on Accreditation of Hospitals. The Council's members are the Canadian Hospital Association, the Canadian Medical Association, the Royal College of Physicians and Surgeons of Canada, and L'Association de Médecine de Langue Française du Canada.

The aim of the program is the same as that of the Joint Commission on Accreditation of Hospitals, to help improve the quality of care rendered to patients in hospitals. There will be little or no departure from the program established by the American College of Surgeons and the Joint Commission on Accreditation of Hospitals. The standards for hospital accreditation of the Commission have been adopted by the Council as a basis for accreditation, as well as the survey procedures and report forms.

Department of Health, Education and Welfare

Dr. LEROY E. BURNEY, surgeon general of the Public Health Service, has announced approval of 45 grants, totaling $4,861,944, to help build and equip additional health research facilities in 40 institutions in 26 states. The grants are the final awards under the appropriation for the fiscal year ending June 30. They also mark the completion of the initial three-year, $90 million construction program authorized by the 84th Con-

gress in July 1956. Since that time a total of 496 grants has been awarded to 248 institutions throughout the U.S. and in Hawaii.

In August 1958, the 85th Congress extended the program at the same level for an additional three years.

The Public Health Service has also awarded a $505,000 contract to the Upjohn Company, Kalamazoo, Michigan, to develop, test and manufacture antibiotic and related drugs in the search for compounds effective in the treatment of cancer. This is the first such contract awarded by the Service's Cancer Chemotherapy National Service Center at the National Cancer Institute under a new patent policy which permits a contractor to patent and sell drugs or other chemical agents developed under contract with the Government.

Seventeen countries of Central and South America and Australia will participate in the Public Health Service's international research training program. Begun a year ago, the program provides medical research training in this country for scientists from abroad. With the 17 announced recently, 30 countries are now participating in the program. The 17 new participants are: Argentina, Australia, Brazil, Ceylon, Chile, Colombia, El Salvador, India, Iran, Japan, Mexico, New Zealand, Pakistan, Peru, Philippine Islands, Thailand, Uruguay. Under the program, the Public Health Service provides a basic annual stipend of $4,500, allowances for wives and children, and limited travel funds.

NIH

Dr. ALAN E. TRELOAR, formerly director of research for the American Hospital Association and director of the Hospital Research and Educational Trust in Chicago, has been appointed chief of the Statistics and Analysis Branch, Division of Research Grants, at NIH. He will be responsible for continuing quantitative analyses and evaluations of all research programs supported by NIH.

The first issue of the *Bulletin of Translation from Russian Medical Sciences*, published by the Russian Scientific Translation Program of the Institutes, is now being distributed to the American professional public interested in the clinical and basic medical sciences. Designed to alert scientists to materials ready for their appraisal, it will contain information about the NIH Russian Scientific Translation Program as well as from other programs, both Government and private. The current issue lists cover-to-cover translations of Russian journals; recent translation and publication of specific issues of journals; distribution of translated abstracts; information on reprints; cost of annual subscriptions; and activities of the Foreign Technical Information Center, Department of Commerce, that would be of interest to those in the medical sciences.

Free subscriptions to the *Bulletin of Translation* may be obtained upon request to the Scientific Reports Branch, Division of Research Services, National Institutes of Health, Bethesda 14, Maryland.

Dr. SEYMOUR D. VESTERMARK, who retired recently as chief of the National Institute of Mental Health's Training Branch, died February 22. Recently awarded a special citation for his efforts in the field of psychiatric training by the American Psychiatric Association, Dr. Vestermark initiated in 1949, and administered until retirement, the Institute's entire training program.

Experiment in medical educational television

The University of Pennsylvania School of Medicine, Jefferson Medical College, Temple University School of Medicine, Hahnemann Medical College, and Woman's Medical College of Pennsylvania recently participated in an experiment which linked the five schools by closed-circuit TV. The special telecasts, a series of five lectures by Dr. Joseph W. Spelman, medical examiner for the City of Philadelphia, marked the first time that five medical schools have used the technique of television for joint lecture-

demonstration purposes. Produced by the Medical Education Service Unit of Smith Kline & French Laboratories, the programs were watched by approximately 1300 students on screens as large as 4½-by-6 feet in their own auditoriums. Subject of the lectures was "Forensic Medicine," the application of medical knowledge to purposes of the law. A telephone hook-up was used to relay the students' questions directly to Dr. Spelman through a moderator at each of the schools.

The National Foundation

Announcement was recently made of the availability of fellowships for postdoctoral study by the Foundation. The fellowships, which provide for a stipend in addition to a compensation to the institution of $1250 annually for the support of the Fellow's program, will cover the broad category of research and the clinical fields of preventive medicine, rehabilitation and orthopedics. Information may be received by writing to the Chief, Division of Scholarships and Fellowships, Department of Professional Education, The National Foundation, 800 Second Ave., New York 17, N.Y.

Veterans Administration

Reappointment of Dr. WILLIAM S. MIDDLETON as chief medical director of the Veterans Administration was announced recently. He has held the post since March 1955, and his new term of office will also be four years. As chief medical director, Dr. Middleton heads the agency's 171 hospitals, outpatient clinics and nationwide hometown medical services, which together provide care for some 2½ million patients yearly.

President Eisenhower recently approved an authorized capacity of 125,000 beds for the VA hospital system. Sumner G. Whittier, administrator of veterans affairs, said the new policy calls for: continuance of complete care for veterans with service-connected disabilities; within the 125,000 bed authorization, continued care for nonservice-connected veterans unable to defray the expense of hospitalization; and authority for the Administrator to shift beds or hospitals, from one type use to another in keeping with medical progress.

PERSONNEL EXCHANGE

Faculty Vacancies

PEDIATRICIAN: Full time clinical teacher for department with active student and house staff educational program. Person interested in clinical teaching as a career desired. Considerable small group teaching with less emphasis on lectures. Rank and salary dependent on qualifications. Address: V-72.

VIROLOGIST and IMMUNOLOGIST: Research position in medical school for young Ph.D. interested in immunology and virology to cooperate in a research program as well as to pursue individual interests. Salary depends upon qualifications and experience. Opportunity for teaching. Address: V-73.

PSYCHIATRIC SOCIAL WORKERS: Active participation in clinical teaching and in expanding program of services in the department of psychiatry and in pediatrics-psychiatry clinic in eastern university medical school. Excellent opportunity for individuals interested in social work contribution in medical education. Qualifications: Master's degree with psychiatric sequence, and for senior positions experience in supervision or teaching, preferably in psychiatric clinical setting. Send curriculum vitae with application. Address: V-74.

PHYSIOLOGIST OR PHARMACOLOGIST: Teaching and research position in medical school. N.Y.C. area. M.D. or Ph.D. required. Training in neurophysiology desired. Salary based on qualifications and experience. Address: V-75.

PROFESSOR OF PREVENTIVE MEDICINE: The University of Alberta invites applications for the position of professor and head of the department of preventive medicine in the faculty of medicine. Duties will include administration of the department, teaching of graduate and undergraduate students and a program of research. Salary will be $10,000 per annum with consulting privileges. Interested applicants should send a complete curriculum vitae, names of three referees, and a recent photograph to the office of the Dean of Medicine, University of Alberta, Edmonton, Alberta.

PEDIATRICIANS: Two or 3 part-time teaching positions —may use remainder of time for private practice. Address: F. G. Gillick, M.D., Creighton University School of Medicine, Omaha 2, Nebraska.

PSYCHIATRISTS: Full-time and part-time teaching positions. Need coordinator of teaching grant program. Would function under departmental director. Address: F. G. Gillick, M.D., Creighton University School of Medicine, Omaha 2, Nebraska.

ASSISTANT PROFESSOR OF PREVENTIVE MEDICINE: Full-time appointment in department of preventive medicine with teaching and research opportunity, including comprehensive medical care teaching. Must have M.P.H. degree. Address: V-76.

FELLOW IN VIRUS RESEARCH: M.D., with at least one year of residency in pediatrics for training in diagnostic virology. Duties include approximately three hours of ward rounds, and five hours of training and research in the virus laboratory. The individual is expected to direct and consolidate activities in the clinical and research areas. Salary $6,000 per annum. Position available for 2-year tenure. Apply Dr. H. A. Wenner, University of Kansas Medical Center, Kansas City, Kansas.

IMMUNOCHEMIST OR BIOCHEMIST: Must be interested in field of infectious diseases. Activities include studies on immune mechanisms and on the biochemistry of virus infections. Full-time research position. Salary open; minimal $8,000. Apply H. A. Wenner, M.D., Section for Virus Research, University of Kansas School of Medicine Kansas City, Kansas

PSYCHIATRISTS: Newly organized department of psychiatry in east coast medical school needs full-time psychiatrists for research and teaching. Rank and salary are dependent on experience and qualifications. Address: V-77.

DIRECTOR OF MEDICAL EDUCATION: for new 350-bed hospital. New position. Internal medicine specialist preferred for full-time position. Beginning salary about $15,000 per year. Address inquiries to: Sister Administrator, Providence Hospital, Washington 17, D.C.

PHARMACOLOGIST: The University of Alberta invites applications for the position of Associate Professor of Pharmacology, effective not later than September 1, 1959, at a minimum salary of $8,000 per annum, with annual increments. Duties include undergraduate and graduate teaching and a program of research. Applicants possessing a medical degree, in addition to qualifications in pharmacology, will be given preference. Applications should include a recent photograph or snapshot, a curriculum vitae, and the names of three references, and should be sent to the Dean of Medicine, University of Alberta, Edmonton, Alberta, Canada.

PSYCHIATRIC SOCIAL WORKER: Position open on new child psychiatry inpatient service to be opened on July 1. Research and teaching will be emphasized in developing program. Preferred qualifications are Ph.D. degree or doctoral candidate with solid clinical grounding in psychiatric social work. Master's degree in Social Work with 5 years psychiatric clinical experience will be considered. Position carries academic appointment. Send curriculum vitae with application. Address: V-78.

To aid in solution of the problem of faculty vacancies, MEDICAL EDUCATION will list persons and positions available, as a free service. The school department or person may have the option of being identified in these columns or of being assigned a key number for each position listed. Mail addressed to key numbers will be forwarded to the person or department listing the request.

Information for these columns should reach the Personnel Exchange, Journal of Medical Education, 2530 Ridge Avenue, Evanston, Illinois, not later than the 10th of the month which precedes the month in which the listings will appear.

Personnel Available

ALLERGIST: Board eligible in medicine. Desires career type opportunity in teaching and research. Has basic training in immunology. Will consider full-time, geographic full-time and half-time opportunities. Address: A-375.

INTERNIST-BIOCHEMIST: Ph.D., M.D. Age 42. Desires opportunity to do research with some clinical work, interested in rheumatic diseases experienced teacher and investigator. Wide scientific background, including radioisotopes, publications. Address: A-376.

ROTATING INTERN: Age 26. Publication co-author. Desires faculty appointment in general surgery. Excellent references. Available July 1959. Address: A-377.

PEDIATRICIAN: Diplomate American Board of Pediatrics. Currently assistant professor; seeking a teaching position in a new location. Address: A-378.

ORTHOPEDIC SURGEON: British, age 36. F.R.C.S. (Edin.) F.R.C.S. (Eng.) Guy's Hospital Medical School, London. Publications, British Medical Journal. Eight years experience. Desires position in American medical school, preferably in orthopedic and traumatic surgery. Prepared to sit any necessary licensure or other examinations. Prefers settling in a maritime state with a warm climate. Address: A-379.

MICROSCOPIC ANATOMIST: M.D., male. Presently associate professor but desires change of locale to upper midwest or west for reasons of health. Seeking academic or research position in medical center or research laboratory. Address: A-380.

PHYSIOLOGIST: Ph.D., 1958, married, three children. Research in circulatory and respiratory physiology. Nine publications; teaching experience with medical and dental students. Desires teaching appointment in New England or New York State, with opportunity for research. Address: A-381.

INTERNIST: Female, age 32; Mayo trained with an interest in hematology. Desires teaching position. Address: A-383.

SURGEON: Age 33, certified general, experience in thoracic and extra-corporeal techniques. University training. Seeks full-time academic position with opportunity for research. Address: A-384.

BIOSTATISTICIAN: Seeking position as member of team in basic medical research or as lecturer to medical, dental, pharmacy and graduate students. Address: A-385.

PEDIATRICIAN: MPH, desires teaching and/or research position with clinical emphasis. Address: A-386.

ANATOMIST: Position wanted in university anatomy department in U.S., by married male with family. British medical school degree in medicine, extensive clinical experience, and recent teaching and research experience in anatomy in England. Good references available. Available to attend interviews in the U.S. now. Address: A-387.

OPHTHALMOLOGIST: Research scientist in field of vision and ophthalmology desires teaching position in Canada or U.S. Long experience in field and extensive publications. Contracts pending and in hand. Address: A-388.

OBSTETRICIAN-GYNECOLOGIST: Foreign physician, age 30, three years residency in obstetrics and gynecology in teaching hospitals of U.S.A., with good command of English, desires position as preceptor in Ob-Gyn., starting July 1959. Address: A-389.

UROLOGIST: Foreign physician, age 30, one year internship, three years in urology and one in urological research in teaching hospitals of U.S.A. Desires position as preceptor in urology, starting July 1959. Good command of English. Address: A-390.

THORACIC SURGEON: M.D. 1947, University of Istanbul. Served an internship in surgery (1954–55) Montana Deaconess Hospital, Great Falls, Montana. Served as Fellow in Thoracic Surgery (sponsored by American College of Chest Physicians) Knoxville, Tenn. Presently in charge of thoracic surgery department at Armenian Hospital, Istanbul. Desires teaching position in American medical school. Address: A-391.

PATHOLOGIST: Age 35, married. Certified PA 1955. Academic background and three years teaching experience. Wishes to relocate in West. Will consider part-time or full-time teaching appointment. Especially interested in surgical pathology. Address: A-392.

OTOLARYNGOLOGIST and HEAD and NECK SURGEON: Age 32; board eligible. University of the Philippines graduate. Completed five and one half years' training in eastern medical centers (3 years otolaryngology, 6 months more bronchoesophagology, and 2 years general and head and neck surgery). Desires one year fellowship, or assistantship, or academic position. Available August or October, 1959. Address: A-393.

BIOCHEMIST: Ph.D., age 30. Assistant professor of biochemistry desires academic position. Five years medical and graduate teaching experience. Membership in national societies, honors, grants, graduate students. Fifteen full-length publications. Research interests: enzymology, microbial metabolism and protein metabolism. Available July 1, 1959. Address: A-394.

PHYSIOLOGIST-PHARMACOLOGIST: Ph.D., 1954. Male, married, with family. Presently teaching physiology in dental school. Desires teaching position with research opportunities in physiology or pharmacology department. Address: A-395.

BIOSTATISTICIAN: Age 43; Ph.D. (mathematics and statistics). One year post-doctoral work in statistics; sixteen years experience in teaching and research in schools of medicine and public health. Desires position doing teaching and/or research. Address: A-396.

PATHOLOGIST-BACTERIOLOGIST: M.S., B.S. (London University); M.R.C.S. (England) L.R.C.P. (London). Age 42, family; registered with British General Medical Council. Five years experience in general and clinical pathology and bacteriology, London, England. Completing 3-year contract in Jamaica. Desires academic appointment in U.S., preferably in the South. Available May, 1959. Address: A-397.

VIROLOGIST-PATHOLOGIST: Excellent experience and background in infectious diseases, human and animal viruses. Broad interests include cancer and pathogenesis. D.V.M.–Ph.D., age 34. Presently in industry. Desires research and teaching position. Would consider Senior Fellowship. Address: A-398.

INTERNIST-HEMATOLOGIST: Age 36, Board certified, with five years academic-type practice and previous research experience, seeks academic position in moderate sized city. Address: A-399.

PSYCHIATRIST: Female, age 26, completing final year of residency in June 1959. Training includes two years in an active university program and participation in family studies in schizophrenia. Analytically (Sullivanian) oriented. Special interests: Psychotherapy with schizophrenics, teaching professionals and non-professionals, liberal arts. Seeks position teaching in medical school with time for limited private practice. Interested in small university community. Address: A-400.

PHARMACOLOGIST: Ph.D., 1955; married, 3 children. Presently teaching pharmacology to medical students. Publications. Research interests: drug metabolism and toxicology. Desires teaching appointment in medical school that would provide opportunity for completion of courses leading to M.D. degree. Would continue teaching pharmacology after receiving the degree. Available August 1. Address: A-401.

INTERNIST: M.D. Age 33. Currently on faculty of eastern medical school. Experience in private practice and industrial medicine. Eight months experience and training in psychiatry. Desires faculty appointment with opportunity for clinical investigation in cardio-vascular diseases, as well as teaching general medicine, in teaching hospital. Address: A-402.

PATHOLOGIST: Wishes to join Pathology Department to contribute to, or help develop, a strong, well balanced teaching program with emphasis on best utilization of materials, methods and communication with students. Also interest and experience in basic research. Previous experience in undergraduate, graduate, and medical teaching, including several years in Anatomy (Ph.D.), followed by M.D., internship and specialization in Pathologic Anatomy (will complete July '59). Age 38. Desires permanent position with good opportunities in primary fields of interest. Address: A-403.

VETERINARIAN: Experienced in microbiological techniques; presently at a medical school. Desires position as director of experimental animal laboratory. Address: A-404.

MICROBIOLOGIST-VIROLOGIST: Ph.D., presently on university faculty. Five years experience in virology and tissue culture publications. Desires academic position involving full-time research or research and teaching. Address: A-405.

ORTHOPAEDIC SURGEON: 38. Wants appointment in U.S.A. or Canada. Main interest in Traumatic Surgery and Research. Now holding consultant post at well known British Hospital. Mastership in Surgery and Fellow of the Royal College of Surgeons. Address: A-406.

INDEX TO ADVERTISERS

Individual Membership

in the

Association of American Medical Colleges

In recent years the activities of the Association of American Medical Colleges have expanded far beyond the original considerations of administrative problems to the many and varied problems of medical education as encountered by the entire medical school faculty.

The expansion of activities has been due to the growing complexity of medical education —the swift development of the medical sciences, the rapid accumulation of new knowledge to be taught, the pressure for more graduates, the changing patterns of medical care, and countless other factors.

Because of these factors, the AAMC recognizes the need for a professional organization to represent not only the medical schools but the faculty members of these schools. Through the offering of individual membership, the AAMC provides you with the opportunity to exchange ideas, opinions and information through the Annual Meeting, Teaching Institutes, and other activities of the Association.

The AAMC also encourages you to attend the Annual Meeting, not only to meet with others who are teaching in your field and discussing the educational problems that are peculiar to it, but also with the idea of becoming familiar with the entire field of medical education as one of society's most important enterprises. The time has come when teachers of medicine must meet together and discuss the problems and activities that are peculiar to medicine as education just as they are accustomed to meet and talk about medicine as science.

As an Individual Member you are entitled to receive *The Journal of Medical Education*, the only publication devoted exclusively to medical education. The Journal also carries the latest news from the medical schools and provides a valuable service through its Personnel Exchange column. You receive the yearly *Directory*, the Proceedings of the Annual Meetings, and *The Medical Monitor*, a newsletter which will keep you informed on items of current interest in the field of medical education, both nationally and internationally.

Individual Membership, at only $10 a year, is open to any person who has demonstrated a serious interest in medical education over a period of years. All the privileges of membership and a provisional membership card are granted immediately after payment of the $10 fee, although confirmation must await official action at the next Annual Meeting.

To obtain membership, fill out the application form below, append check for $10, and return to the Association's central office at 2530 Ridge Ave., Evanston, Ill.

INDIVIDUAL MEMBERSHIP APPLICATION

ASSOCIATION OF AMERICAN MEDICAL COLLEGES

2530 Ridge Ave., Evanston, Ill.

Name: _____

Mailing Address: _____

_____

(City) (Zone) (State)

Field of medical education in which chief interest lies: _____

_____

College or other connection: _____

_____

NOW many more hypertensive patients may have THE FULL BENEFITS OF CORTICOSTEROID THERAPY

Except for one case of mild blood-pressure elevation (150/90) **no hypertension was seen in any of 1500 patients**† as a result of treatment with DECADRON—the new and, on a milligram basis, most potent of all corticosteroids. **Hypertension induced by other steroids diminished or disappeared.**

Thus with DECADRON, hypertension no longer appears to be a contraindication to successful corticosteroid therapy. And the dramatic therapeutic impact of DECADRON was virtually unmarred by diabetogenic or psychic reactions . . . Cushingoid effects were fewer and milder . . . and there were no new or "peculiar" side effects. Moreover, DECADRON helped restore a "natural" sense of well-being.

†Analysis of clinical reports.

Decadron*

DEXAMETHASONE

treats more patients more effectively

*DECADRON is a trademark of Merck & Co., Inc. ©1959 Merck & Co., Inc.

MERCK SHARP & DOHME
DIVISION OF MERCK & CO., Inc., PHILADELPHIA 1, PA.

Sobee® *formula was selected...*

Robert ... *age 4 months*

Bobbie was a first child, and his mother didn't know what to do when he became a "crying baby," shortly after coming home from the hospital. He cried between feedings, and even *during* feedings. Then his doctor suggested a 2 day trial of Sobee ... and the crying stopped! Now you've never seen such a bright, *cheerful* baby.

Willa ... *age 5 months*

Like all allergic babies (like all *babies*), Willa needs love and lots of it. Willa gets love, in full measure, from her parents—while Sobee keeps her happily nourished.

Thomas ... *age 7 months*

Tommy at 1 month was a colicky baby, irritable, usually crying—but physically normal except for an all-over rash. When Sobee replaced Tommy's milk formula, the crying stopped. His skin improved in a few days, and the rash was completely gone in a few more. Tommy liked Sobee, grew well on it. About a month ago Tommy was "weaned" back to milk, uneventfully.

SOBEE® (HYPOALLERGENIC SOYA FORMULA, MEAD JOHNSON) LIQUID/POWDER

Mead Johnson

Symbol of service in medicine

FP2455M